Rubinstein, Leonard Jay

THE ACTIVATION OF SILENT CLONES AND THE IDIOTYPE REGULATION OF THE GROWTH OF ABPC48 PLASMACYTOMA CELLS

City University of New York PH.D. 1985

PLEASE NOTE:

In all cases this material has been filmed in the best possible way from the available copy. Problems encountered with this document have been identified here with a check mark __✓__.

1. Glossy photographs or pages __✓__
2. Colored illustrations, paper or print _____
3. Photographs with dark background __✓__
4. Illustrations are poor copy _____
5. Pages with black marks, not original copy _____
6. Print shows through as there is text on both sides of page _____
7. Indistinct, broken or small print on several pages __✓__
8. Print exceeds margin requirements _____
9. Tightly bound copy with print lost in spine _____
10. Computer printout pages with indistinct print _____
11. Page(s) _____ lacking when material received, and not available from school or author.
12. Page(s) _____ seem to be missing in numbering only as text follows.
13. Two pages numbered _____. Text follows.
14. Curling and wrinkled pages _____
15. Dissertation contains pages with print at a slant, filmed as received _____
16. Other _____

_____

_____

University
Microfilms
International

THE ACTIVATION OF SILENT CLONES AND THE IDIOTYPE REGULATION OF THE GROWTH OF ABPC48 PLASMACYTOMA CELLS

by

LEONARD JAY RUBINSTEIN

A dissertation submitted to the Graduate Faculty in Biomedical Sciences in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

1985

This manuscript has been read and accepted for the Graduate Faculty in Biomedical Sciences in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

07. 30. 85
date

Constantin A. Bona, M.D., Ph.D.
Chairman of Examining Committee

1/30/85
date

Terry A. Krulwich, Ph.D.
Executive Officer

Jerome L. Schulman, M.D.

James G. Wetmur, Ph.D.

Patricia K. A. Mongini, Ph.D.

Kathryn E. Stein, Ph.D.

Supervisory Committee

City University of New York

ABSTRACT

THE ACTIVATION OF SILENT CLONES AND THE IDIOTYPE REGULATION OF THE GROWTH OF ABPC48 PLASMACYTOMA CELLS

by

Leonard Jay Rubinstein

Advisor: Professor Constantin A. Bona

The treatment of newborn BALB/c mice with minute amounts of either the A48 myeloma protein or monoclonal proteins that express A48 idiotopes, followed by immunization with bacterial levan 2-4 weeks later, elicits an anti-levan response that is dominated by the A48Id. The expansion of $A48Id^+$ B cell clones in these mice is associated with an increase in A48Id-specific helper T cells. The passive influx of maternal idiotypes into the fetus also exerted profound effects on the development of the immune repertoire. Antibodies displaying a particular idiotype can indirectly expand clones expressing the corresponding idiotype by first stimulating idiotype-specific helper T cells. The study of genetic control of the expression of the A48Id has clearly shown that its expression is not dependent upon MHC or IghC gene complexes in newborn mice treated with either anti-A48, A48, or hyperimmunized with anti-A48-KLH conjugates. However, the activation of U10Id B cell clones through in utero exposure to UPC10 myeloma protein, is dependent on $IghV^a$ genes. The neonatal administration of anti-A48 antibodies causes a long-lasting activation of $A48Id^+$ bearing clones which is related to a direct interaction of the anti-A48Id antibodies and the immune receptor of B cell clones that are $A48Id^+$. The maturation of these precursors requires challenge by antigen, but antigenic challenge can be replaced by a monoclonal anti-A48Id antibody,

thereby representing a homobody, and carrying the internal image of the antigen. The effect of antigen, activation of normal A48Id$^+$ B cell clones, and monoclonal antibodies sharing A48-UPC10 regulatory idiotopes on the in vivo growth of ABPC48 myeloma cells was also examined. Immunogenic doses of bacterial levan have no detectable effects, whereas tolerogenic doses substantially delay the growth of the myeloma. The activation of normal A48Id$^+$ B cell clones also has no apparent effect on the growth of the ABPC48 myeloma cells. Among a panel of 15 monoclonal antibodies expressing A48-UPC10 regulatory idiotopes, and expressing V_H genes derived from the V_H441-4 germ line gene family, 5 were able to provide a long lasting, but not definitive idiotype specific protection against the ABPC48 myeloma cells.

ACKNOWLEDGEMENTS

It is with sincere appreciation that I acknowledge my mentor Constantin Bona. His academic motivation and laboratory guidance throughout my training at Mount Sinai has provided me with a firm understanding of cellular immunology. This training was of the highest caliber and will invariably be utilized in my future career as a research scientist.

I would like to acknowledge all my friends who were part of my graduate experience at Sinai. Carol, remember when we were the only two working in 16-70 and the discussions on idiotype. Jeff, thanks for helping out on those long working days in the lab. Audrey, a true friend who was always there when I needed her. Bert, Tony and Blanche, my only regret is that we did not work together longer.

I acknowledge Carmen Rodriguez for her expert preparation and typing of this dissertation. If not for the long hours she spent at the typewriter, completion would not have been possible.

Finally, I acknowledge my parents and sister who throughout my academic career supported me through the emotional ups and downs. It is through their support that I have been able to pursue the Ph.D. degree.

TABLE OF CONTENTS

INTRODUCTION..... 1

MATERIALS AND METHODS..... 18

Animals..... 18

Antigens..... 18

Myeloma proteins and monoclonal antibodies..... 18

Purification of monoclonal antibodies..... 20

Preparation of anti-idiotype antisera..... 21

Labeling of sheep erythrocytes (SRBC) with BL, In, TNP and monoclonal antibodies..... 21

Iodinated antigens..... 21

Iodine, tritium and alkaline phosphatase labeling of monoclonal antibodies..... 22

Determination of serum antibody titers to BL, In and TNP..... 22

Determination of serum idiotype titers..... 23

Determination of shared A48 idiotypes by enzyme linked immunosorbent assay (ELISA)..... 24

Isoelectric focusing (IEF)..... 25

Detection of plaque forming cells (PFC)..... 25

Purification of B and T cells..... 26

Immunization schedules of mice receiving myeloma transplantation..... 26

Spleen adaptation of ABPC48 ascitic myeloma cells..... 27

Monitoring of myeloma growth..... 27

Determination of anti-A48 and anti-AIDA23/3 antibodies in the sera of mice immunized with various monoclonal antibodies expressing A48-UPC10 regulatory idiotopes..... 29

RESULTS

Activation of silent clones by treatment at birth with idiotypes..... 31

Occurrence of A48Id-bearing immunoglobulins in BALB/c mice treated at birth with A48 monoclonal protein..... 31

Occurrence of A48Id molecules was not related to persistence of A48 monoclonal protein injected at birth..... 31

Ontogeny of the A48Id response..... 34

Antigen specificity of activation of A48Id clones..... 37

Effect of BL-binding monoclonal antibodies sharing the A48Id..... 39

The inability of purified B cells to transfer the $A48Id^+$ response..... 41

Expansion of A48Id-specific helper T cells..... 44

Effect of injection of the UPC10 myeloma protein during pregnancy on the expression of $U10Id^+$ plaque forming cells in progeny..... 48

Genetic control of activation of A48Id silent clones after manipulation of the immune network..... 52

Genetic control of activation of A48Id silent clones by anti-A48Id antibodies..... 52

Genetic control of activation of A48Id silent clones by administration at birth of A48Id monoclonal antibodies..... 55

Genetic control of activation of U10Id silent clones by in utero exposure to U10Id monoclonal antibodies..... 57

The requirement for immunization with antigen or monoclonal anti-idiotypic antibodies for the activation of β2→6 and β2→1 polyfructosan-reactive clones in BALB/c mice treated at birth with minute amounts of anti-A48 idiotype antibodies..... 57

Requirement of antigenic challenge for the activation of the $A48Id^+$ response in BALB/c mice treated at birth with anti-A48Id antibodies..... 57

Long-lasting activation of precursors of $A48Id^+$ antibody-producing cells after treatment at birth with anti-A48Id antibodies..... 61

A monoclonal anti-A48Id antibody is able to replace the antigenic challenge required for the activation of an anti-β2→6 fructosan response..... 63

Analysis of IgG antibodies by IEF..... 69

T independence of the activation of A48Id$^+$ clones after treatment at birth with anti-A48Id antibodies..... 72

Regulation of myeloma growth by antigen and regulatory idiotopes..... 76

Kinetics of myeloma growth..... 76

Effects of antigen immunization on the growth of ABPC48 myeloma cells..... 79

Effect of activation of normal A48Id$^+$ B cell clones on the growth of ABPC48 myeloma cells..... 81

Effect of monoclonal antibodies expressing A48-UPC10 regulatory idiotopes on the growth of ABPC48 myeloma cells..... 81

Idiotype specificity of anti-myeloma immunity..... 87

DISCUSSION..... 91

BIBLIOGRAPHY..... 109

LIST OF TABLES

1. Genetic characteristics of mice used in this study..... 19

2. Presence of serum antibodies specific for BL..... 32

3. Ontogeny of the A48Id response..... 36

4. Specificity of activation of A48Id silent clones..... 38

5. Ability of three β2→6 fructosan-binding monoclonal antibodies to adsorb the binding capacity of anti-A48Id antibodies..... 40

6. Treatment at birth with different monoclonal antibodies..... 42

7. Failure to transfer A48Id response with B cells from 1-mo-old mice treated at birth with A48 monoclonal proteins..... 43

8. Ability to transfer $A48Id^+$ response in lethally irradiated BALB/c mice with syngeneic T cells from mice treated at birth with A48 monoclonal protein..... 45

9. Demonstration of A48Id-specific helper T cells in BALB/c mice treated at birth with 10 μg A48 monoclonal protein: Helper effect on anti-TNP antibody response in nude mice..... 47

10. Effect of the maternal transfer of UPC10 on the Anti-BL response in progeny..... 50

11. Demonstration of U10Id specific helper T cells in progeny originating from BALB/c mice injected during pregnancy with UPC10 myeloma protein..... 51

12. Activation of A48Id silent clones by the administration of 10 ng anti-A48Id monoclonal protein at birth..... 54

13. Activation of A48Id silent clones by hyperimmunization of adult mice with anti-A48-keyhole limpet hemocyanin..... 56

14. Activation of A48Id silent clones by the administration of 10 μg of A48Id monoclonal protein at birth..... 58

15. Activation of U10Id silent clones by in utero exposure to U10Id monoclonal protein..... 59

16. Requirement of antigen for the activation of the $A48Id^+$ response in BALB/c mice treated at birth with anti-A48Id antibody..... 62

17. Long-lasting activation of the $A48Id^+$ response after treatment at birth with anti-A48Id antibody..... 64

18. Ability of 17-38 monoclonal anti-A48Id antibody to elicit an anti-BL PFC response in mice treated at birth with 0.01 μg of anti-A48Id antibody..... 65

19. Age dependence and the specificity of activation of anti-β2→6 and anti-β2→1 fructosan clones in BALB/c mice treated at birth with 0.01 μg anti-A48Id antibody and challenged with 17-38 monoclonal antibody..... 68

20. Transfer of $A48Id^+$ response with B cells from mice treated at birth with 0.01 μg of anti-A48Id antibody..... 77

21. Relationship between the number of myeloma cells injected and the growth of the myeloma..... 78

22. Effect of immunogenic and tolerogenic doses of antigen on the kinetics of myeloma growth..... 80

23. Effect of normal $A48Id^+$ B cell clones activated by the administration at birth of minute amounts of anti-A48Id antibodies on myeloma growth..... 82

24. Summary of immunochemical and molecular properties of monoclonal antibodies expressing A48-UPC10 regulatory idiotopes..... 83

25. Titers of anti-A48Id and anti-AIDA23/3 in the sera of immunized mice..... 85

26. Effect of monoclonal antibodies expressing A48-UPC10 regulatory idiotopes on the growth of ABPC48 myeloma cells..... 86

27. Determination of the permanence of anti-myeloma immunity..... 88

28. Idiotype specificity of anti-myeloma immunity..... 89

LIST OF ILLUSTRATIONS

1. Kinetics of the clearance of [^{3}H] A48 injected intraperitoneally at day 0..... 33

2. Dose-effect relationship of treatment of newborn mice with various doses of A48 monoclonal protein..... 35

3. Dose-effect relationship of treatment of newborn mice with various doses of anti-A48Id polyclonal antibody..... 60

4. Dose-effect relationship of the challenge with various doses of 17-38 monoclonal anti-A48Id antibodies..... 67

5. Serum from individual, 1-month-old BALB/c mice analyzed by IEF autoradiography using ^{125}I-BL antigen overlay..... 70

6. Serum from individual, 3-month-old BALB/c mice analyzed by IEF autoradiography using a ^{125}I-In-BSA antigen overlay..... 71

7. Serum from individual, 3-month-old BALB/c mice analyzed by IEF autoradiography using a ^{125}I-In-BSA antigen overlay..... 73

8. Serum obtained from individual CXBJ and BALB/c mice after immunization with BL and analyzed by IEF autoradiography using a ^{125}I-In-BSA antigen overlay..... 74

9. Serum from an individual CXBJ mouse obtained before and after immunization with BL and analyzed by IEF autoradiography with various antigen overlays..... 75

10. Functional dualism of a homobody..... 103

INTRODUCTION

It has been three decades since Slater et al. (1955) first demonstrated among 21 human sera from patients with multiple myeloma, that every one of the myeloma proteins studied were immunologically different, indicating individual antigenic specificities. However, since myeloma proteins arise from a malignant state, it was believed possible that the individual antigenic determinants which the myeloma proteins bore were markers for the products of malignant cells rather than normal constituents of an antibody molecule.

Several years later Kunkel et al. (1963) showed that individual human anti-blood group antibodies will elicit secondary antibodies in the rabbit which are specific for the antibody. Furthermore, the specificity obtained was found directed only against the individual antibody used for immunization. At the same time Oudin and Michel (1963) described a similar phenomenon with rabbit anti-Salmonella typhi antibodies.

In 1964 Gell demonstrated that rabbit antisera generated against rabbit antibodies specific for Proteus vulgaris cells, reacted only with the antibody preparation used as the immunogen.

Grey et al. (1965) presented data which determined the site on the myeloma protein to which this individual specificity was localized through a study of various fragments obtained by reductive and enzymatic cleavage of these proteins. The antigenic determinants responsible for the individual specificity were localized in all cases studied solely to the Fab fragment produced by papain digestion. Furthermore, individual specificity could be localized to the heavy chain of the Fab fragment (Fd) or isolated light chains of the Fab

fragment.

Oudin and Michel (1969a,b) extended their studies with rabbit antibodies against S. typhi and introduced the term "idiotypy" to define this specificity that was restricted (a) to a single antibody, and (b) to a single individual.

The documentation up until this point suggested that idiotypic determinants were unique antigenic determinants which were borne solely by a nonspecific population of antibodies within one individual. However, Kunkel (1970) noticed that when rabbit anti-idiotypic antisera were produced to several human IgM monoclonal cold agglutinins, the sera complied with the criteria for being anti-idiotypic because there was no reaction with normal human IgM antibodies. However, the sera did react with other IgM cold agglutinins. Thus, the cross-reactive idiotypic specificity (IdX) was defined as that determinant which is shared only by antibodies of related specificities.

Several murine myeloma proteins were observed to share cross-reactive idiotypic determinants with a series of antibodies which share the antigenic specificity of the myeloma proteins. Potter and Lieberman (1970) demonstrated that the majority of all BALB/c anti-phosphorylcholine antibodies shared a cross-reactive idiotype with the BALB/c myeloma protein TEPC15. This myeloma protein is specific for phosphorylcholine. Similarly, Blomberg et al. (1972) observed that more than 85% of the BALB/c α,1-3 dextran specific antibodies share a cross-reactive idiotype expressed on three BALB/c myeloma proteins specific for dextran, J558, MOPC-104E and U102. Lieberman et al. (1976) described three distinct cross-reactive idiotypes using a panel of 13 inulin-binding myeloma proteins.

Some idiotypic determinants are closely associated with the combining site of the antibody molecule. Thus, the interaction of idiotype and anti-idiotype can be inhibited by the antigen. Brient and Nisonoff (1970) described this phenomenon in the rabbit arsonate idiotype system. It was noted that benzoate haptens inhibited the anti-idiotype from precipitating radiolabeled idiotype. Lieberman et al. (1975) demonstrated that many of the idiotypes displayed by the inulin-binding myeloma proteins were inhibitable by $\beta 2 \rightarrow 1$ fructosan trisaccharides.

However, not all idiotypic determinants are located in the combining site of immunoglobulins. Spring-Stewart and Nisonoff (1973) generated an anti-idiotypic serum to rabbit anti-arsonate antibodies, by immunizing rabbits with immune complexes of these antibodies and arsonate. Thus, idiotypes located in the combining site would be unexposed. The result was an anti-idiotypic sera which serologically was defined as anti-idiotypic, non-reactive with normal antibodies, but the reaction between idiotype and anti-idiotype was simply not antigen inhibitable.

The question arises as to whether idiotypic determinants can be found on isolated heavy and light chains, or if an interaction of both heavy and light chains is needed for idiotype expression. In the human system Grey et al. (1965) demonstrated that idiotypic determinants could be located on individual heavy or light chains. However, in most of the murine systems studied, the interaction of a particular heavy and light chain is required for full expression of the idiotype. Lieberman et al. (1977) constructed recombinant molecules in the inulin idiotype system. They found that the variable region of the light

chain (V_L) determined which idiotype was to be expressed, but that expression only occurred when it was bound to a variable region of a heavy chain (V_H) of another inulin-binding myeloma protein.

Antibodies which share idiotypic determinants often show strong homology in the amino acid sequence at various segments within the variable region. Kunkel et al. (1973, 1974) had completely sequenced the V_H and V_L domains of two human monoclonal IgM proteins which had anti-IgG activity and shared a cross-reactive idiotype. The V_H domains differed by only 8 out of 120 positions, and 7 of the 8 differences occurred in framework residues (Capra and Kehoe, 1974a,b). The two V_L domains were assigned to different light chain subgroups, and had 31 amino acid differences between them. However, the sequences in the second and third hypervariable regions on the two light chains shared perfect sequence homology (Capra et al., 1976). From these results it would appear that idiotypy in this case was determined by the amino acid sequence of the second and third hypervariable regions of the heavy and light chains.

The induced inulin specific antibodies of BALB/c mice were studied by Vrana et al. (1976) for homology between idiotypy and amino acid sequence. It was previously demonstrated that the light chain determined the idiotypy in the inulin idiotype system (Lieberman et al., 1977). The variable region of the heavy chain of four inulin-binding myeloma proteins was sequenced. The results demonstrated that they differed only by 6 out of 120 residues. All these 6 differences though were associated with the framework regions. Sequence information indicated that one of the cross-reactive idiotypes was formed by the association of two amino acids in the first and third

hypervariable region.

In 1974 Niels Jerne proposed a theory on the regulation of immune responses based on the information available to him at that time. This novel and fundamental idea would become one of the most important contributions to our understanding of immunology in the past decade, and was termed the network theory of the immune system. In his proposal Jerne introduced some new terminology. He proposed to replace the term "antigenic determinant" by the term "epitope", and the term "antibody combining site" by the term "paratope". Thus, epitopes and paratopes complement one another like a lock and key. Jerne expanded on the observations made by Oudin and Michel, and described an idiotype as a set of epitopes displayed by the variable regions of a set of antibody molecules. Each single idiotypic epitope he referred to as an "idiotope". Therefore, an idiotype represents a certain set of idiotopes. Thus, antibodies have a functional dualism, namely that antibody molecules can recognize as well as be recognized. These properties led to the establishment of a network, and as antibody molecules occur both free and as receptor molecules on lymphocytes, this network intertwines cells and immunoglobulins. Through the paratope-idiotope interactions within the system, the immune system achieves a dynamic steady state as its elements interact between themselves.

Jerne's network regulation was based on idiotypic communication between clones in response to antigen upsetting the steady state of the network. The basis for this concept came from experiments presented by Hart et al. (1972), in which they demonstrated that by injecting A/J mice with a rabbit anti-idiotype directed to the major cross-reactive

idiotype borne by arsonate specific antibodies of A/J mice, they could specifically obliterate that portion of the arsonate specific response without altering the overall magnitude of the response. Minute amounts of antibody exerted the desired suppressive effect on a very specific group of cells, namely, those bearing the idiotype.

Let us now consider how a functional immune network would be described for a foreign antigen which presents epitope e to the immune system. This epitope is recognized by a set of paratopes p1 which bind the epitope with varying degrees of affinity. These paratopes are associated with a set of idiotopes i1. Therefore, p1i1 represents the set of antibody molecules and potentially responding lymphocytes with the immune receptor p1i1, with respect to the epitope e. Continuing along the same lines, each paratope of the set p1 recognizes a larger set of idiotopes. This set of idiotopes, i2, represent the "internal image" of the epitope e, since both are recognized by p1. The set of idiotopes i2 is associated with a set of paratopes p2.

There is a set of paratopes which recognize i1 called p3. Jerne referred to this group of antibody molecules as anti-idiotypic. Lindenmann (1979) redefined the "internal image set" as "homobodies". Thus, the paratopes represent the negative image of the epitopes, and the idiotopes fitting a given paratope and resembling the positive image of the epitopes at which that paratope is directed, the "homobody". One cannot clearly differentiate between occupation of a paratope by another paratope (belonging to anti-idiotype) directed at an idiotypic configuration close to or even overlapping the paratope, and occupation of a paratope by idiotope (belonging to homobody). Thus, the distinction between homobodies and anti-idiotypic antibodies

is difficult to obtain. Recently Jerne et al. (1982) classified anti-idiotype antibodies into two categories. Ab2α, the anti-idiotype antibodies directed against the conventional idiotype of the antibody which binds the foreign epitope, and Ab2β, the anti-idiotype antibodies carrying the internal image of the antigen.

In order to study a functional immune network a suitable system must be obtained. The immune network formed by the clones that comprise the bacterial levan idiotype pathway displays certain characteristics that render it particularly suitable for studies of regulation of the immune response. Immunization with bacterial levan (BL), which is a β2→6 polyfructosan with β2→1 branch points, leads to a vigorous T cell-independent antibody response in adult mice (Miranda, 1972). This response in BALB/c mice is composed of two distinct families of antibodies. The first binds β2→6, whereas the second binds β2→1 fructosan epitopes. This latter group also shares the dominant cross-reactive idiotype with the inulin-binding myeloma proteins (Lieberman et., 1975). Inulin is a polyfructosan made up of β2→1 linkages. In the response to levan, there is a substantial ontogenic delay in the appearance of clones specific for β2→1 linkages, and they do not appear until 8 weeks of age (Bona et al., 1978). However, the β2→6 specific clones can be expanded at birth, and therefore studied independently of the anti-β2→1 fructosan response in one month old animals. Antibodies bearing the idiotopes expressed on ABPC48 (A48) and UPC10, two independent β2→6 fructosan-binding myeloma proteins, cannot be identified in the sera of BL-immune mice, suggesting that the A48 idiotype (A48Id) or UPC10Id are markers of silent or minor clones (Lieberman et al., 1975).

Oudin and Cazenave (1971) reported some very puzzling data which seemed to contradict the earlier definition of an idiotype. They demonstrated that antibodies produced against different non-cross-reactive epitopes of one foreign antigen may have similar idiotypes.

In 1981 Bona et al. suggested that although antibodies to conventional antigenic determinants express many idiotopes, only a limited number of these idiotopes function in eliciting responses in an autologous or syngeneic system. They designated these idiotopes as "regulatory idiotopes".

The concept of the regulatory idiotope emerged from the study of the antigen binding properties of various members of an idiotype network pathway (Bona et al., 1981). A limited immune network has been identified, in which there are four distinct members. A series of anti-idiotype antibodies consisting of Ab2 (anti-idiotype), Ab3 (anti-anti-idiotype), and Ab4 (anti[anti(anti-idiotype)]) were induced by immunization of syngeneic mice with the A48 β2→6 fructosan-binding myeloma protein (Ab1). The hyperimmunization of syngeneic mice with Ab1 induces the synthesis of anti-A48Id antibodies. However, syngeneic mice immunized only twice with Ab1 often leads to the production of anti-(anti-A48Id) antibodies. Similarly, hyperimmunization with Ab2 also leads to the synthesis of Ab3. Finally, hyperimmunization with Ab3 leads to the appearance of anti-[anti-(anti-A48Id)] antibodies. This chain of anti-idiotype antibodies exhibits an interesting feature. Both Ab1 and Ab3 share a cross-reactive idiotype since they are both recognized by Ab4 and Ab2. However, the affinity of Ab4 for Ab1 is lower than that of Ab2 for Ab1. The network conformed by these antibodies is asymmetrical, because only Ab1, and not Ab3 recognizes

bacterial levan.

Wikler et al. (1979) demonstrated a similar limited network working with rabbit anti-idiotypic antibodies in the Micrococcus lysodeikticus and in the tobacco mosaic virus systems. The most outstanding features of their results were that Ab4 antibodies could recognize specifically Ab1, thus Ab4 behaves like Ab2, and that diversity did not increase along the chain of immunization. Thus, Ab4 was the limit to the length of the anti-idiotypic network that could be detected.

Gleason and Kohler (1982) generated T helper cells in BALB/c mice by phosphorylcholine immunization. Their data indicated that idiotype specific T cells could recognize a common antigenic determinant found on both TEPC15-negative and TEPC15-positive myeloma proteins. TEPC15 is the major idiotype expressed in BALB/c mice in response to immunization with phosphorylcholine. This shared determinant was determined to be located in the binding site for phosphorylcholine.

Goldberg et al. (1983) screened a sample of 198 mouse and 80 human myeloma proteins for the expression of the A48Id. Among these proteins they found one, MOPC167, a BALB/c myeloma protein which expressed A48 idiotopes but had an affinity for phosphorylcholine.

A48 and UPC10, both of which are β2→6 fructosan-binding myeloma proteins share idiotype expression. This is not surprising, since both are members of the V_HIII subgroup, and nucleotide sequences of the expressed genes from these two proteins, indicate a substantial homology among amino acid residues 32 to 97 of the V_H region (Auffray et al., 1981). The UPC10 V_H gene appears to be derived from the V_H 441-4 germ line gene family (Ollo et al, 1981). MOPC173, another

BALB/c myeloma protein with unknown antigen binding specificity also is derived from the V_H441-4 germ line gene, and expresses some idiotopes in common with A48 and UPC10. The D and J segments of A48, UPC10 and MOPC173 however are quite distinct from one another (Ollo et al., 1981).

In summary, regulatory idiotopes represent a special set of idiotopes which are characterized by several criteria (Bona, 1984). One, they can function as autoimmunogens and are able to induce the synthesis of auto-anti-idiotype antibodies. Two, they should be shared by several members of an idiotypic pathway and could be shared by antibodies with different antigen binding specificities. Three, the clones expressing these idiotopes possess the potential of becoming dominant, possibly because regulatory T cells specific for regulatory idiotopes are present prior to immunization or may be elicited in the course of an immune response.

Although the A48Id is normally silent in BALB/c mice, under certain experimental conditions, it can be induced to appear in an anti-levan immune response. Athymic nude BALB/c mice treated with anti-E109IdX antibodies and challenged with bacterial levan, leads to a suppression of the E109IdX$^+$ response with a concomittant appearance of A48Id$^+$ antibodies (Bona et al., 1979b; Lieberman et al., 1979). E109 is the dominant cross-reactive idiotype present on inulin-binding myeloma proteins and expressed in an anti-levan immune response. In BALB/c mice treated at birth with minute amounts (10 ng) of anti-A48Id antibodies, and immunized one month later with bacterial levan, approximately 40% of the anti-levan antibody forming cells will be secreting antibodies which are A48Id$^+$ (Hiernaux et al., 1981). Adult

BALB/c mice hyperimmunized with anti-A48Id antibodies develop a substantial $A48Id^+$ anti-levan response after immunization with bacterial levan (Bona, et al., 1981). The ability to manipulate such a network could turn out to be useful in the control of the growth of myelomas.

The recognition of myelomas as spontaneous or induced malignant transformations of antibody-secreting cells provided a tool that has proven to be of enormous value. The use of homogeneous antibodies produced by myelomas, both human and murine, was essential for the elucidation of immunoglobulin structure and eventually the discovery of idiotypy.

The activation of antigen-specific lymphocytes into effector cells initiated by the interaction of antigens with specific receptors on the lymphocyte, inititiated Burnet (1959) to propose the clonal selection hypothesis. As a natural extension of this hypothesis, it follows that an individual must also be unresponsive (tolerant) to self antigens.

In 1971 Sirisinha and Eisen reported that antibodies made by BALB/c mice to myeloma proteins from mouse plasmacytomas of BALB/c origin were found to be specific for the idiotypic determinants of the myeloma proteins. Shortly thereafter, Lynch et al. (1972) demonstrated that immunization with the purified myeloma protein M315, rendered mice resistant to challenge with otherwise lethal numbers of MOPC315 myeloma cells. The resistance to myeloma transplantation was observed to be idiotype specific. Thus, immunization with M315 did not render mice resistant to another myeloma, namely MOPC460, which has the same antigen binding specificity as MOPC315, but expresses a different

idiotype. Those studies, in effect, established that the idiotypes on myeloma proteins could function as tumor-specific transplantation antigens.

That myeloma proteins could function as tumor-specific transplantation antigens has been demonstrated in other systems as well. Meinke et al. (1974) demonstrated that mice immunized with S121 or MOPC-104E myeloma proteins were resistant to challenge with lethal numbers of either S121 or MOPC-104E myeloma cells respectively.

Eisen et al. (1975) studied a panel of 7 myeloma proteins and their ability to provide resistance. Of the four anti-phosphorylcholine myeloma proteins tested, MOPC167 was particularly immunogenic and required only a small amount (10 μg) to generate an anti-idiotypic response. The mice immunized with this protein and challenged with a lethal number of cells were resistant to transplantation. The MOPC167 myeloma is more malignant than most murine plasmacytomas (TD_{50} 600 cells).

Freedman et al. (1976) demonstrated that preimmunization of mice with purified M component of MOPC-11 resulted in significant relative immunity to subsequent challenge with 10^4 viable MOPC-11 myeloma cells. However, if they challenged with either 10^5 or 10^6 viable cells, immunity was overcome and the mice died.

Hannestad et al. (1972) demonstrated that murine myeloma cells had surface membrane localized myeloma protein. They suggested that its presence at the cell surface created a potential target for immune effectors. It is surprising however that secreted myeloma protein did not neutralize the cellular and humoral idiotype-specific effectors responsible for resistance. If anything, secreted myeloma protein

could help the myeloma cells escape effector mechanisms by providing a buffering zone of soluble idiotype.

It is well known that normal plasma cells arise from the differentiated lineage of non-secretory B lymphocytes. Tumors are supposed to represent cells immortalized at a particular stage in differentiation. Thus, myelomas should consist of a homogeneous population of plasma cells. However, what if myeloma cells were heterogeneous, and that they differentiated during in vivo growth. In 1977 Rohrer et al. set out to test this hypothesis. They monitored the growth of MOPC315 myeloma cells suspended in diffusion chambers which were implanted in the peritoneal cavity of BALB/c mice. What they observed was that cells of the BALB/c myeloma MOPC315 could differentiate in vivo as normal B cells did. Differentiation was manifested by a progressive change from small, stem cell-rich, non-secretory lymphocytoid cells to larger, M315Id-secreting plasmacytoid cells. Thus, idiotype-specific myeloma transplantation resistance might be mediated at the level of a non-secretory myeloma cell.

Antigen-driven differentiation of normal B cells has been well documented (Williamson et al., 1976). It was of interest to note if myeloma cells were also sensitive to these same signals. Antigen-driven differentiation of normal B cells for the hapten TNP (trinitrophenyl), requires interactions between the TNP-immunogen, T cells, macrophages, and the immunoglobulin-producing B cells.

Rohrer and Lynch (1977) observed that MOPC315 growth and differentiation were stimulated when the myeloma cells were enclosed with TNP-SRBC (sheep red blood cells) in peritoneal diffusion chambers implanted into mice in whom SRBC-specific T helper activity had been

induced. However, if the mice were immunized with a dose of SRBC which induced T suppressor activity, MOPC315 growth and differentiation were suppressed. Furthermore, if the hapten was coupled to a carrier other than SRBC, growth and differentiation were also suppressed. These results established that carrier-primed T cells were necessary for the regulation of MOPC315 growth and differentiation.

Antigens may also have a negative effect on the growth of myeloma cells. Abbas and Klaus (1977) demonstrated that IgA secretion by MOPC315 myeloma cells could be inhibited in vitro by culturing the cells with various DNP (dinitrophenyl)-carrier conjugates. Suppression of antibody production was specific, non-IgA proteins and DNA synthesis remained unaffected. Inhibition only occurred with haptenated gamma-globulins, and the use of other carriers such as bovine serum albumin was also ineffective. Inhibition could be reversed if the antigen was removed from culture.

Paraf et al. (1975) demonstrated they could inhibit the growth of the murine plasmacytoma TEPC15 in vivo by treatment with the specific pneumococcus C polysaccharide antigen. Mice were injected intraperitoneally with the TEPC15 myeloma cells and 10 μg of the antigen. This was followed by treatments with the antigen twice a week for 4 or 6 weeks. Tumor growth was inhibited by 35 to 60% using this protocol.

Bhoopalam et al. (1980) demonstrated that the murine plasmacytomas MOPC-104E and J558, which secrete antibodies specific for α,1-3 dextran, could be inhibited in vivo in BALB/c mice receiving a single intraperitoneal injection of 10 μg of α,1-3 dextran into the mice from 7 days before and up to 3 days after the transplantation of the

plasmacytoma cells.

As previously mentioned, immunization of BALB/c mice with purified BALB/c myeloma protein rendered them resistant to challenge with otherwise lethal numbers of homologous myeloma cells. Frikke et al. (1977) in an attempt to identify the mechanism that mediated idiotype-specific suppression of myeloma, failed to observe a constant relationship between the quantity of anti-idiotype antibody induced and the degree of resistance to MOPC315 challenge.

It had been established that the thymus played a major role in immunoregulation (Gershon, 1975). Daley et al. (1978b) observed that thymectomy performed after immunization with M315 had been completed, resulted in a total abrogation of resistance to MOPC315 challenge. Adoptive transfer studies with thymocytes lended evidence to support the hypothesis that T cells in M315Id immune mice specifically regulate MOPC315 differentiation and subsequent resistance.

Abbas et al. (1980b) showed that after intravenous immunization of BALB/c mice with MOPC315 immunoglobulin-coupled syngeneic cells, the nylon wool purified splenic T cells isolated from these animals could induce a 40 to 70% suppression of antibody secretion by the myeloma cells, as assessed by plaque assays and [^{3}H]leucine incorporation. These suppressor cells could be absorbed out on and eluted from plastic petri dishes coated with the M315 myeloma protein. Likewise, Bona and Paul (1979) have demonstrated naturally occurring suppressor T cells specific for the idiotypic determinants of the myeloma MOPC460.

As a more complete understanding of the molecular and cellular mechanisms that mediate immunoregulation of murine myeloma cells

become available, it may become possible to therapeutically manipulate human myeloma cells by regulation of their differentiation. Indeed, there are a few reports of applying the current understandings of immunoregulation on human malignant lymphomas.

In 1982 Miller et al. first demonstrated with a single patient that they could enzymatically remove the immunoglobulin receptor from the surface of lymphoma cells isolated from their patient with a non-Hodgkins B cell lymphoma. They then used these antibody molecules to generate anti-idiotype antibodies in mice. After treatment of the patient with the mouse monoclonal anti-idiotype antiserum, the patient showed a remarkable and complete remission after 42 months of the lymphoma.

More recently Meeker et al. (1985b) treated 10 patients with B lymphocytic malignancy with anti-idiotype therapy. As before these monoclonal anti-idiotype antibodies were prepared in mice, and five of the ten patients have had objective remissions which were also clinically significant. However, these remissions were not complete and were of relatively short duration.

These new strategies for developing specific therapeutic protocols are very promising, but as yet not perfected. Further investigation into the mechanism of immunoregulation of myeloma cells by the host and through network manipulations will provide a better approach for future therapy.

In the next section I will present the data collected on the manipulation of the A48 functional immune network. In the first section I will present the data on an idiotype-induced activation of silent clones and the cellular components involved in the activation.

Data on the mechanism responsible for the activation of silent clones through anti-idiotype, as well as the genetic restriction of these responses will also be discussed.

The second section of this dissertation will investigate the role of a functional immune network on the in vivo growth and development of the BALB/c plasmacytoma ABPC48. The parameters discussed will include the effects of antigen, activation of normal B cell clones by anti-idiotype which express the same idiotype as ABPC48, and the effect various monoclonal antibodies bearing regulatory idiotopes have on the in vivo growth and development of the ABPC48 myeloma cells.

MATERIALS AND METHODS

Animals. Normal and nude BALB/c mice were purchased from the Charles River Breeding Laboratory, Wilmington, MA. One-day-old mice were obtained from our breeding colonies at the Mount Sinai School of Medicine, New York, NY. CCB.R4 mice were a gift from Dr. R. Riblet at the Institute for Cancer Research, Philadelphia, PA. BALB.B, BALB.K, BAB.14 and CAL.20 mice were a gift from Dr. M. Potter, Laboratory of Cell Biology, National Cancer Institute, NIH, Bethesda, MD. The recombinant inbred strain CXBJ was obtained from The Jackson Laboratory, Bar Harbor, ME. Characteristics of the mice used are illustrated in Table 1.

Antigens. Bacterial levan (BL) was isolated by alchohol precipitation from culture supernates of Aerobacter laevenicum (ATCC15552) grown at 23°C in nutrient broth supplemented with 5-10% sucrose. Trinitrophenyl-aminoethylcarbamylmethyl-Ficoll (TNP-Ficoll) was prepared according to the method of Inman (1975). Inulin (In) was obtained from the Calbiochem-Behring Corp., San Diego, CA. It was coupled to Brucella abortus (In-BA) according to the method of Chien et al. (1979). TNP-protein conjugates, such as TNP-A48, were prepared by the method of Bikoff (1982). Protein-keyhole limpet hemocyanin conjugates, such as A48-KLH, were prepared according to the method of Bona et al. (1979a). Type 3 pneumococcal polysaccharide (S-III) was a gift from Dr. P. Baker, Laboratory of Microbial Immunity, NIAID, NIH, Bethesda, MD.

Myeloma proteins and monoclonal antibodies. The BALB/c myeloma proteins used in this study were ABPC48 (A48), UPC10 (U10), W3082, MOPC384 (M384), RPC5, LPC1, MOPC460 (M460) and MOPC173 (M173). A48,

TABLE 1

Genetic Characteristics of Mice Used in this Study

Mouse Strain	Major Histocompatibility Complex of the Mouse	IghV	IghC
BALB/c	d	a	a
BALB.B	b	a	a
BALB.K	k	a	a
BAB.14	d	a	b
CCB.R4	d	b	a
CAL.20	d	d	d

W3082, M384 and M460 are all IgA immunoglobulins, whereas U10, RPC5, LPC1 and M173 are all IgG2a immunoglobulins. A48 and U10 possess β2→6 fructosan-binding activity. W3082 has a binding affinity for inulin. M460 is a dinitrophenyl (DNP)-binding myeloma protein. M384 myeloma protein binds Salmonella tranaroa lipopolysaccharide. RPC5, LPC1 and M173 myeloma proteins have an unknown binding specificity. These myeloma proteins were kindly provided by Dr. M. Potter, Laboratory of Cell Biology, NCI, NIH, Bethesda, MD.

The monoclonal anti-M460Id antibody, CD5.3 was a gift from Dr. J.F. Kearney, The Comprehensive Cancer Center, University of Alabama in Birmingham, Birmingham, AL. Several monoclonal antibodies were prepared from three experimental protocols designed to activate $A48Id^+$ silent clones. Series one (e.g. 1-5-1) (Goldberg et al., 1983) were obtained from animals primed at birth with 10 μg A48 followed one month later by immunization with BL. Series two (e.g. 2-1-3, etc.) were obtained from animals primed at birth with 10 ng anti-A48 followed one month later by immunization with BL. Series three (e.g. 3-27-6, etc.) were obtained from animals hyperimmunized with anti-A48-KLH in Freund's complete adjuvant (FCA) followed by immunization with BL. Four monoclonal Ab3 antibodies (e.g. AIDA10/16, etc.) originating from BALB/c mice immunized with syngeneic monoclonal anti-A48Id antibodies were also used. These monoclonal Ab3 antibodies were kindly provided by Dr. P. Legrain, Unité de Génétique Somatique, Département d'Immunologie, Institut Pasteur, Paris, France.

Purification of monoclonal antibodies. All IgM monoclonal antibodies were purified on a sephacryl 300 column in Tris buffer pH 8.0. All IgA monoclonal antibodies were purified in the same way

except for A48. A48 was affinity purified on an IDA10-sepharose column. All the IgG monoclonal antibodies were purified by first precipititating ascites fluid with 45% ammonium sulfate, and then running the antibodies on a DEAE column.

Preparation of anti-idiotype antisera. Anti-A48Id antisera were prepared in BALB/c mice by immunization with an A48-KLH conjugate. Anti-M460Id, anti-U10Id and anti-W3082Id antisera were prepared in the same way. The schedule used for immunization has been previously described (Bona et al., 1979a). Several syngeneic monoclonal anti-A48Id antibodies were also obtained from a fusion of immune lymphocytes from BALB/c mice immunized with A48Id and the nonsecreting myeloma cell line Sp2/0-Ag14 (Shulman et al., 1978).

Labeling of sheep erythrocytes (SRBC) with BL, In, TNP and monoclonal antibodies. An o-stearoyl derivative of BL and In were prepared according to the method of Hammerling and Westphal (1967). SRBC (Pocono Rabbit Farm, Canadensis, PA) were washed three times with saline and resuspended to a final concentration of 10% v/v. The reaction vessel contained 1 ml of 10% SRBC, 3 ml saline and 10 µl stearoyl levan. The mixture was allowed to incubate for 30 minutes at 37°C with gentle agitation. The SRBC were then washed three times with saline to stop the coupling reaction and remove any uncoupled levan. The same procedure was used for coupling In to SRBC. TNP-SRBC were prepared according to the method of Rittenberg and Pratt (1969). SRBC were coupled with monoclonal antibodies by the chromic chloride method (Gold and Fudenberg, 1967).

Iodinated antigens. Inulin-bovine serum albumin (In-BSA) was prepared by the method of Chien et al. (1979) and iodinated

(^{125}I-In-BSA) by the chloramine T method (Greenwood et al., 1963) to a 30 μCi/μg specific activity. BL was tyraminated by the method of Keck (1977) and iodinated (^{125}I-BL) by the chloramine T method to a 10 μCi/μg specific activity.

Iodine, tritium and alkaline phosphatase labeling of monoclonal antibodies. Monoclonal antibodies were iodinated by the chloramine T method (Greenwood et al., 1963). Monoclonal antibodies were tritiated by the method of Wilder et al. (1979). Iodinated goat anti-mouse immunoglobulin was obtained commercially from New England Nuclear (Boston, MA).

For alkaline phosphatase labeling, a 5 mg sample of alkaline phosphatase suspended in 3.2 M ammonium sulfate, pH 7.0 (Sigma Chemical Co., St. Louis, MO), was sedimented in a micro-centrifuge for 5 minutes at 4°C. The supernatant was discarded and 1 ml of purified antibody (2 mg/ml) in phosphate buffered saline (PBS) was added. This mixture was dialyzed overnight against PBS at 4°C. Following dialysis, 20 μl of 10% glutaraldehyde was added to the enzyme-antibody mixture and allowed to react for 2 hours at room temperature. At the end of 2 hours, the mixture was dialyzed overnight against PBS at 4°C, followed by dialysis against 0.05 M Tris-buffer (pH 8) containing 0.001 M magnesium chloride added as a stabilizing agent. The conjugated antibody was then resuspended to a final volume of 5 ml with the same Tris-buffer containing 0.5% BSA.

Determination of serum antibody titers to BL, In and TNP. The passive hemagglutination assay (HA) was employed for the evaluation of titers of antibodies to BL, In and TNP. The assay was carried out on a 96 U-bottom well flexible assay plate (Falcon 3911). Each well

received 25 µl of 3% fetal calf serum (FCS) in saline. Then 25 µl of the antiserum was added to the first well of each row and twofold serial dilutions were made, followed by an additional drop (25 µl) of 3% FCS. Then 25 µl of 1% SRBC coupled with the appropriate antigen was added. The plates were covered with sealers and allowed to sit at room temperature for approximately 3 hours. The titer recorded is given as $1/\log_2$ of the highest dilution of antisera giving agglutination.

A radioimmunoassay (RIA) was also employed for the determination of antibodies to BL, In and TNP. Flexible assay plates were coated with either 50 µl of BL (50 µg/ml), In-BSA (30 µg/ml) or TNP-BSA (30 µg/ml) and incubated for 18 hours at 4°C. The plates were then washed three times with PBS and incubated for 3 hours at 4°C with various dilutions of immune sera. After washing again with PBS, the plates were incubated with ^{125}I-goat anti-mouse (50,000 cpm/50 µl) for 3 hours at 4°C. Plates were extensively washed with PBS, individual wells cut, and counts measured in a gamma counter (Beckman Biogamma). The concentration of anti-BL, anti-In, and anti-TNP antibodies in the sera of immunized animals was determined from the linear portion of a standard curve obtained with known amounts of unlabeled UPC10, W3082, and IgM-anti-DNP monoclonal antibodies respectively.

Determination of serum idiotype titers. A hemagglutination inhibition assay (HI) was used to determine the titers of serum A48Id and M460Id in immunized animals. The assay was carried out on a 96 U-bottom well flexible assay plate. Each well received 25 µl of 3% FCS. Then 25 µl of immune serum was added to the first well of each row and twofold serial dilutions were performed. This was followed by

the addition of 25 µl of purified anti-idiotype antibody, at a minimal concentration known to induce hemagglutination. Finally, 25 µl of 1% SRBC coupled with the appropriate idiotype, namely A48 or M460 myeloma protein, was added. The titer recorded is expressed as $1/\log_2$ of the highest dilution of sera giving inhibition.

An RIA was also performed to determine the titer of idiotypes in the sera of immunized animals. The serum level of A48Id was determined by coating microtiter plates with affinity purified anti-A48Id antibodies (50 µg/ml) and ^{125}I-A48 as the ligand. The precise concentration of antibody expressing the A48Id was determined from a standard inhibition curve obtained with A48 myeloma protein. The serum level of antibodies bearing the M460Id was determined by coating the plates with monoclonal antibody CD5.3 (3 µg/ml) and ^{3}H-M460 as the ligand. The precise concentration of M460Id bearing molecules was determined from a standard inhibition curve obtained with unlabeled M460 myeloma protein.

Determination of shared A48 idiotypes by enzyme linked immunosorbent assay (ELISA). Microtiter plates were coated for 18 hours at 4°C with A48, U10, 3-76-36, or 3-76-42 (50 µg/ml) followed by 3 washings with PBS. The plates were then incubated for 3 hours at 4°C with alkaline phosphatase labeled, affinity purified syngeneic anti-A48Id antibodies (5 µg/50 µl) to establish the extent of binding of the anti-A48Id antibodies to individual monoclonal antibodies. To confirm the sharing of idiotypes, the alkaline phosphatase labeled anti-A48Id antibodies were preabsorbed for 30 minutes at room temperature with equal amounts of purified A48, U10, 3-76-36, or 3-76-42 monoclonal antibodies. After extensive washing, the plates were incubated with

substrate for 1 hour at 37°C, and the reaction was measured at 405 nm on a Dynatech ELISA reader.

Isoelectric focusing (IEF). IEF was performed by a modification of the method described by Briles and Davie (1975) and refined by Nicolotti et al. (1980). Further details of the procedure are described by Stein et al. (1980).

Detection of plaque forming cells (PFC). The number of PFC secreting antibody specific for BL, In, or TNP was determined by two modifications of the Jerne and Nordin (1963) plaque assay. In the first technique 50 µl of a spleen cell suspension were mixed with 50 µl of 10% SRBC coated with either BL, In or TNP, in 0.3 ml of 0.5% agarose at 45°C. This mixture was then spread evenly over a glass slide previously coated with 0.1% agar. The slides were incubated for 2 hours at 37°C in the presence of RPMI 1640 media; this was followed by a 1 hour incubation at 37°C in the presence of guinea pig complement diluted 1:20 with Dulbecco's PBS. To determine the number of anti-BL PFC secreting antibody carrying A48, U10, and W3082 idiotypes, anti-In PFC carrying the W3082Id, and anti-TNP PFC carrying the M460Id, anti-A48, anti-U10, anti-W3082, and anti-M460 antiserum in a final dilution of 1:10,000, 1:5,000, 1:100, and 1:500, respectively, were added to the agarose at the time of plating on the glass slides. The number of PFC observed in the presence of anti-idiotype was subtracted from the number observed if no inhibitor was present. The difference was considered to be the number of PFC secreting anti-BL, anti-In, or anti-TNP antibody expressing the corresponding idiotypes.

Similarly, the Cunningham and Szenberg (1969) modification of the plaque assay was also used. Two glass sides were taped together with

double stick tape to create a channel between the two slides. A mixture containing, 50 µl spleen cell suspension, 10 µl 10% SRBC, 10 µl guinea pig complement diluted 1:3, and when appropriate 10 µl of anti-idiotype antiserum, was applied to the channel. The slides were then sealed with parrafin and incubated for 1 hour at 37°C. The number of PFC were counted at this time.

Purification of B and T cells. Purified splenic B lymphocytes were obtained by injecting mice intraperitoneally with 0.2 ml rabbit anti-mouse thymocyte serum (M.A. Bioproducts, Walkersville, MD) 2 days before being sacrificed. The splenic lymphocytes were harvested and treated with anti-Thy1.2 antisera plus complement to remove any residual T cells.

Purified splenic T lymphocytes were collected by two alternate methods. First, plastic petri dishes (100 x 15 mm) were coated with 3 ml of rabbit anti-mouse immunoglobulin serum (ammonium sulfate fraction, 1 mg/ml) for 1 hour at 37°C. The plates were then washed twice with PBS, and 3 ml of a spleen cell suspension (5×10^7 cells) was added. Plates were incubated at room temperature for 1 hour with gentle agitation every 15 minutes. After 1 hour, the nonadherent spleen cells were harvested from the plates. Alternatively, splenic T lymphocytes were collected by passage of splenic lymphoctyes over nylon wool columns.

Immunization schedules of mice receiving myeloma transplantation. All mice received a total of 1 mg of the immunizing antibody divided into five weekly injections of 200 µg. The first injection was in FCA, the second was in Freund's incomplete adjuvant (FIA), and all subsequent injections were in PBS; at first injection, the antigen was

distributed in rear footpads and four subcutaneous sites on the abdomen of the animal. The subsequent injections were distributed in the same four subcutaneous sites. Mice received the transplantation of the myeloma cells 10 days after the last injection.

Spleen adaptation of ABPC48 ascitic myeloma cells. The ascitic form of ABPC48 was adapted to grow in the spleen by the following protocol. Mice were injected intravenously with 1×10^8 viable ascitic ABPC48 myeloma cells. After 7-14 days, the spleens from these animals were harvested and minced into a single cell suspension. These cells were then injected intravenously into another set of mice (1×10^8 cells/mouse), and the procedure was continued until adaptation occurred. This process took about 6 months. Spleen adapted MOPC315 myeloma cells were kindly provided by Dr. R. Lynch, Department of Pathology, College of Medicine, The University of Iowa, Iowa City, IA.

Monitoring of myeloma growth. Four criteria were used to monitor the growth of the ABPC48 myeloma:

a) survival - the average length of time the animals survived after injection with ABPC48 myeloma cells;

b) spleen weight - due to the homing and proliferation of the adapted myeloma cells in the spleen, spleen weights increased from 4 to 10 fold over normal spleens;

c) [^{3}H] thymidine incorporation - this procedure took advantage of the highly proliferative nature of the myeloma cells. Spleen cells (1×10^5) were cultured in 0.2 ml RPMI 1640 media supplemented with glutamine and antibiotics, for 4 hours at 37°C with 0.1 μCi [^{3}H] thymidine. After the 4 hours, the cells were harvested onto filter paper discs and counted in a Beckman LS9000 liquid scintillation

counter. Typical counts were about 100 to 300 fold above background;

d) titer of levan binding IgA in the serum - the titer of levan binding IgA in the serum of experimental animals was determined by two alternate methods. The first was an RIA in which plastic microtiter plates were coated with 50 µl of BL (20 µg/ml) in carbonate buffer (pH 9.6) for 1 hour at 37°C. The plates were then washed 3 times with PBS and 50 µl of 1% BSA was added to each well and incubated for 30 minutes at room temperature. Plates were washed again with PBS and the diluted sera were added to the wells. After 18 hours at 4°C, the plates were washed, and 50 µl of ^{3}H-anti-IgA (25,000 cpm/50 µl) was added. Plates were incubated for 18 hours at 4°C, then extensively washed (15 times), and the individual wells cut up and counted in a liquid scintillation counter. The titer of levan binding IgA in the sera was calculated from the linear portion of a standard curve using A48 myeloma protein. The second method was to use an ELISA assay, in which 100 µl of BL (20 µg/ml) in carbonate buffer was added to each well of the microtiter plate and allowed to incubate for 1 hour at 37° C. Plates were then washed 3 times with PBS containing 0.05% Tween. The diluted sera were then added to the plates and allowed to incubate for 18 hours at 4°C. This was followed by 3 washes with PBS/Tween and then the addition of 100 µl of alkaline phosphatase labeled anti-IgA (diluted 1:15,000). After 18 hours at 4°C, the plates were washed again with PBS/Tween and 100 µl of substrate (1 mg/ml) in diethylamine buffer (pH 8.0) was added. After 45 minutes at 37°C, the plates were read on a Dynatech ELISA reader at an OD of 405 nm. The titer of levan binding IgA in the sera was again determined from the linear portion of a standard curve using A48 myeloma protein as the standard.

Growth of the spleen adapted MOPC315 myeloma cells was assayed using the method of Daley et al. (1978a), in which 14 days after the intravenous injection of 4×10^4 myeloma cells, the spleen is removed, fixed in Bouin's fixative, and the number of plasmacytoma colony forming units (PCFU) of the spleen counted.

Determination of anti-A48 and anti-AIDA23/3 antibodies in the sera of mice immunized with various monoclonal antibodies expressing A48-UPC10 regulatory idiotopes. The titer of anti-A48 antibodies in the sera of immunized mice was recorded from a competitive inhibition RIA or ELISA. Microtiter plates were coated with 50 µl (5 µg/ml) of affinity purified A48 myeloma protein in carbonate buffer, and incubated for 1 hour at 37°C. After incubation the plates were washed 3 times with PBS and 1% BSA was added to the plates for 30 minutes at room temperature. The diluted sera were added next and allowed to incubate for 18 hours at 4°C. The microtiter plates were washed and then ^{125}I-IDA10 (50,000 cpm/50 µl) was added. IDA10 is a monoclonal anti-A48Id antibody. After 2 hours at room temperature, the plates were washed extensively with PBS and the individual wells counted in a gamma counter. The titer was recorded off the linear portion of a standard inhibition curve with unlabeled IDA10. The same procedure was used for the estimation of anti-AIDA23/3 antibodies, by coating the plates with AIDA23/3 and then following the same protocol as above.

The titer of anti-A48Id antibodies was also determined using an ELISA assay. Microtiter plates were coated with 100 µl (10 µg/ml) of affinity purified A48 myeloma protein in carbonate buffer for 1 hour at 37°C. Plates were then washed with PBS/Tween and the diluted sera were added and placed at 4°C for 18 hours. Plates were washed again and

alkaline phosphatase labeled IDA10 (diluted 1:7,000) was added for 6 hours at 4°C. Plates were washed again and 100 µl of diethylamine buffer containing the substrate was added for 45 minutes, and incubated at 37°C. The titer was recorded off the linear portion of a standard inhibiton curve obtained with unlabeled IDA10.

RESULTS

Activation of Silent Clones by Treatment at Birth with Idiotypes

Occurrence of A48Id-bearing immunoglobulins in BALB/c mice treated at birth with A48 monoclonal protein. We are extending our previous observation, which showed that A48Id silent clones can be activated by injection of anti-A48 antibodies, by studying the effect of the administration of BL and A48 monoclonal protein on the expression of these clones at birth. Injection of 10 μg of A48 monoclonal protein at birth, followed 1 month later by immunization with BL, led to the appearance of A48Id-bearing molecules in the serum of these mice, whereas injection of 10 μg of BL alone did not activate the A48Id response. The appearance of $A48Id^+$ molecules in the serum required antigenic stimulation, because mice treated at birth only with A48 monoclonal protein, but not immunized with BL, do not show significant increases in the levels of anti-BL antibodies and A48Id-bearing molecules (Table 2). These results indicated that administration of A48 monoclonal protein at birth led to the activation of clones expressing the A48Id; this activation requires antigenic stimulation.

Occurrence of A48Id molecules was not related to persistence of A48 monoclonal protein injected at birth. The presence of A48Id-bearing immunoglobulins in the serum of mice treated at birth with A48 monoclonal protein can originate in principle from the persistence of protein injected at birth. To investigate this possibility, we injected mice with 13×10^6 cpm of ^{3}H-A48 and studied the clearance of radioactivity from the serum for 34 days by collecting blood samples at various intervals. As can be seen in Fig. 1, the amount of radioactivity measured at 34 days would correspond to $<8 \times 10^{-4}$ μg of

TABLE 2

Presence of Serum Antibodies Specific for BL

Treatment at birth	Immunization 1 mo later	Number of mice studied	Titer log2 units	
			HA (BL)	HI (A48Id)
Nil	Nil	6	1.5 ± 0.8	0.0 ± 0.0
Nil	BL	2	5.5 ± 0.5	0.5 ± 0.5
10 μg BL	BL	3	5.0 ± 0.6	0.0 ± 0.0
10 μg A48	Nil	5	0.8 ± 0.2	0.4 ± 0.2
0.1 μg A48	BL	4	6.0 ± 0.6	3.2 ± 0.2
10 μg A48	BL	7	5.7 ± 0.6	2.7 ± 0.7

Anti-BL and A48Id titers were determined 5 d after immunization with 20 μg BL. The titers recorded are the mean ± SEM.

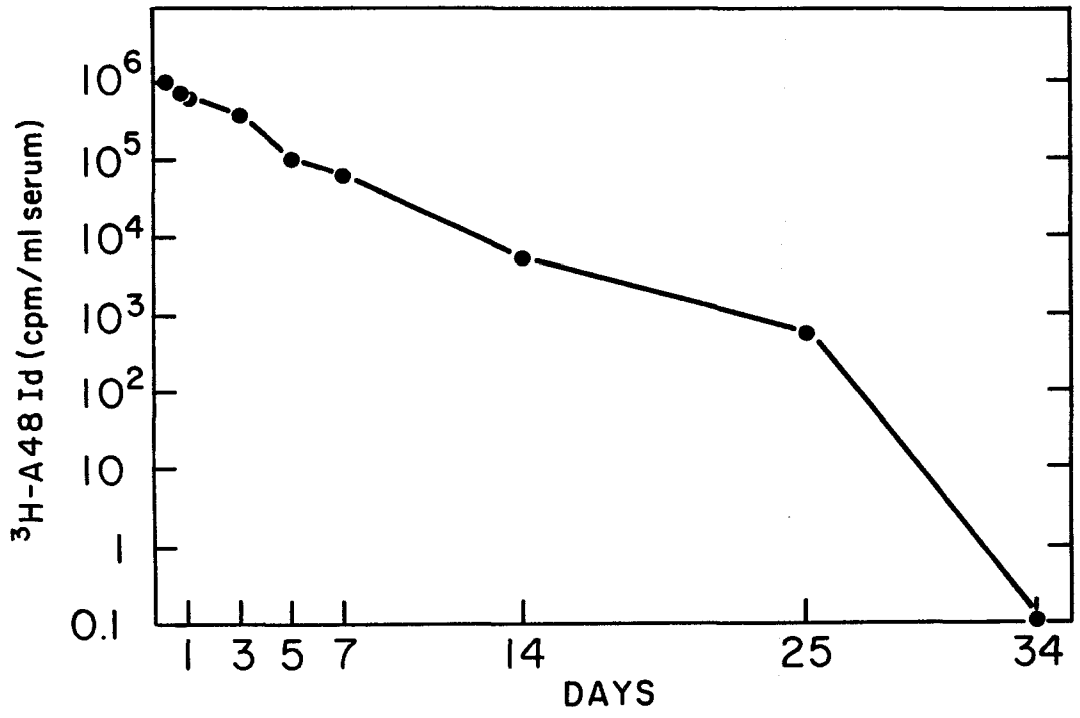

FIGURE 1. Kinetics of the clearance of [^{3}H]A48 injected intraperitoneally at day 0.

A48 monoclonal protein remaining in the circulation from the original injection of 10 μg at birth. Furthermore, we studied the PFC response of mice treated at birth with various amounts of A48 monoclonal protein and immunized 1 month later with BL. The results depicted in Fig. 2 showed that the magnitude of the total direct anti-BL PFC response was not significantly different in the mice whether or not they were treated at birth. However, the A48 component of this response was significantly increased. The combined results regarding the complete clearance of labeled A48 protein 1 month after injection and the possibility of detecting an A48Id$^+$ PFC response demonstrate a genuine activation of A48Id$^+$ clones. The PFC results indicate that A48Id silent clones dominate the anti-BL response in animals treated at birth with A48 monoclonal protein.

Ontogeny of the A48Id Response. The ontogeny of the A48Id response was studied in two groups of animals. The first group was injected at birth with A48 monoclonal protein and immunized either 1, 2, or 3 weeks later with BL. The second group was injected at various intervals after birth with A48 monoclonal protein and immunized 1 month later with BL. The results of this experiment, presented in Table 3, showed that 1-day-old A48-pretreated BALB/c mice immunized 2 or 3 weeks later with BL developed an A48Id response. Similar results were obtained by using a PFC assay (data not shown). In contrast, the animals injected with A48 at various intervals after birth (7-28 days) and immunized 1 month later with BL failed to develop an A48Id response despite a strong anti-β2→6 fructosan antibody response (data not shown). These results show that treatment at birth with A48 monoclonal protein is critical for the activation of A48Id component of the

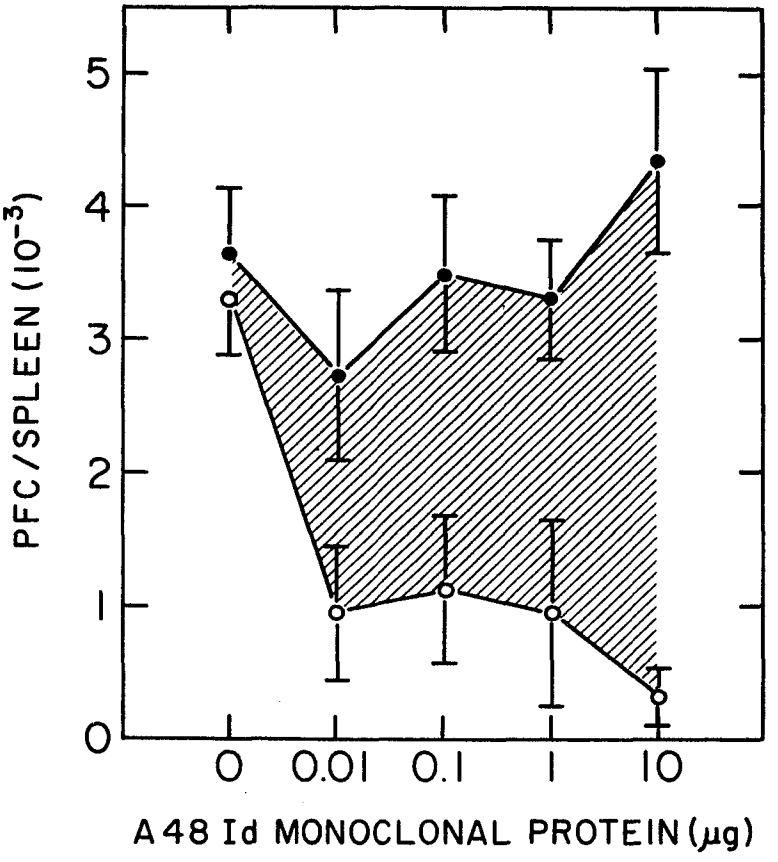

FIGURE 2. Dose-effect relationship of treatment of newborn mice with various doses of A48 monoclonal protein. Mice were 1 day old at the time of treatment and challenged 1 month later with 20 μg BL. (●) PFC/spleen (○) PFC/spleen detected when syngeneic anti-A48Id antisera had been added to the agarose. Shaded area shows the number of PFC bearing A48Id. Each point represents the mean ± SEM of determinations performed on five mice.

TABLE 3

Ontogeny of the A48Id Response

Age of mice			Antibody response: Anti β2→6 fructosan		Idiotype response: A48Id	
A48 treatment	BL immunization	Assay of response	HA[1]	RIA[2]	HI[3]	RIA[2]
	d	d				
Nil	7	12	ND[4]	<0.1	ND	ND
1 d	8	12	ND	1.1 ± 0.1	ND	ND
Nil	14	19	4.3 ± 0.3	1.6 ± 0.2	0.3 ± 0.3	1.7 ± 0.7
1 d	14	19	5.7 ± 0.3	1.2 ± 0.8	1.7 ± 0.3	14.2 ± 3.3
Nil	21	26	4.3 ± 0.7	1.1 ± 0.6	0.0 ± 0.0	1.7 ± 0.1
1 d	21	26	4.7 ± 0.3	1.3 ± 0.7	1.0 ± 0.6	0.4 ± 0.1

Immunization of mice was performed with 20 µg BL intravenously, whereas the treatment at birth was carried out with 10 µg A48 monoclonal protein intraperitoneally.

[1]Titer recorded is mean ± SEM of log2 of the highest dilution of antisera giving agglutination.

[2]Titer recorded is mean ± SEM for of protein in sera (µg/ml).

[3]Titer recorded is mean ± SEM of log2 of the highest dilution of sera giving inhibition.

[4]Not determined.

anti-BL response.

Antigenic specificity of activation of A48Id clones. The specificity of the antigen-dependent activation of A48Id component of the anti-BL response was studied in animals treated at birth with A48 and immunized 1 month later with either BL, In-BA, or TNP-Ficoll as well as in animals treated at birth with M460 and immunized 1 month later with BL and TNP-Ficoll (Table 4). The animals immunized with BL at 1 month of age have a significant increase in the titer of only anti-β2→6 fructosan antibodies and of A48Id component response compared with the mice treated at birth with A48Id monoclonal protein. Anti-β2→6 and anti-β2→1 fructosan antibody levels were slightly increased in mice immunized with In-BA, however, a significant increase of anti-β2→6 and β2→1, and a slight increase of A48Id component was observed in animals treated at birth with A48Id monoclonal protein. These results show that, despite the ontogenic delay of the anti-β2→1 clones that has been previously described (Bona et al., 1978), the administration at birth of A48 monoclonal protein probably stimulated a set of clones that express at least some A48 idiotopes and that secrete antibody able to bind both β2→6 and β2→1 fructosan epitopes. TNP-Ficoll immunization does not affect the activation of $A48Id^+$ clones and, conversely, the M460Id component of the anti-TNP response was not altered by A48 treatment at birth. Furthermore, treatment at birth with M460Id did not activate A48Id component of the anti-BL response. Therefore, our results suggest that the activation of A48Id silent clones is antigen, as well as idiotype, specific. Interestingly, the activation of a set of clones expressing the A48Id reactive to both β2→6 and β2→1 fructosan epitopes was observed in these animals. The existence of these clones

TABLE 4

Specificity of Activation of A48Id Silent Clones

Treatment at birth	Immunization	Antibody response						Idiotype response			
		Anti-β2+6		Anti-β2+1		Anti-TNP		A48Id		M460Id	
		HA	RIA	HA	RIA	HA	RIA	HI	RIA	HI	RIA
Nil	BL	5.7±0.3	5.0±2.6	1.3±0.3	47.7±22.3	2.7±0.3	<3	0	<1	0	0.2±0.2
A48	Nil	3.3±0.8	<0.1	1.3±0.7	<3	2.2±0.5	ND[1]	0.3	<1	0	ND
A48	BL	5.3±0.4	4.6±0.2	0.9±0.4	26.8±16.6	1.0±0.5	<3	2.6±0.4	82.4±47.6	0	0.4±0.1
Nil	In-BA	3.3±0.3	<0.1	2.7±0.3	4.0±0.5	2.7±0.3	<3	0	1.7±0.1	0	0.7±0.4
A48	In-BA	6.0±0.0	1.5±0.7	5.0±1.0	83.0±41.6	1.7±0.9	<3	1.3±0.3	65.0±59.5	0	0.2±0.2
Nil	TNP-Ficoll	1.3±0.7	0.7±0.3	ND	ND	5.7±0.3	>100	0	2.5±1.6	2.3±1.2	ND
A48	TNP-Ficoll	4.3±0.7	2.2±0.1	ND	ND	6.7±0.3	>100	0	3.6±1.4	3.0±1.0	ND
A48	Bl + TNP-Ficoll	7.3±0.3	37.1±21.7	ND	ND	7.3±0.3	>100	3.7±0.3	179.3±63.3	3.0±0.6	1.4±0.8
M460	BL ± TNP-Ficoll	3.2±0.5	3.3±0.7	ND	ND	4.0±0.8	42.2±17.5	0.2±0.2	<1	3.2±0.5	30.4±18.0

HA and HI titers expressed as mean log2 units ± SEM: RIA expressed as mean μg/ml ± SEM. Immunization of 1-mo-old mice treated or not with 10 μg A48 or M460 monoclonal proteins at birth were immunized with 20 μg BL intravenously. 0.1 ml of 1% In-BA intraperitoneally or TNP-Ficoll (20 μg intraperitoneally) respectively. The response was tested 5 d later.

[1]Not determined.

was confirmed by analysis of monoclonal antibodies obtained by fusion of Sp2/0 myeloma cells with spleen cells from BALB/c mice treated at birth with A48 and immunized with BL. Among eight monoclonal antibodies, five were found to bind β2→6 and β2→1 fructosan epitopes (Goldberg et al., 1983).

Effect of BL-binding monoclonal antibodies sharing the A48Id. There are data that suggest that the A48Id of A48 myeloma protein is composed of several idiotopes and that some of them are shared with UPC10, another β2→6 fructosan-binding myeloma (Legrain et al., 1978). Recently, we have obtained four monoclonal antibodies from BALB/c mice producing anti-(anti-A48Id) antibodies that were immunized with BL. These antibodies also express some of the idiotopes of the A48 family, as was assessed by cross-adsorption experiments of anti-A48Id antibodies in the ELISA assay. The results presented in Table 5 showed that only a few idiotopes of A48Id are shared by UPC10, 3-76-36 and 3-76-42 because: (a) the binding of alkaline phosphatase-labeled anti-A48Id antibody to U10, 3-76-36 and 3-76-42 microtiter plates is significantly lower than that of anti-A48 to A48; (b) the binding of anti-A48 antibodies to microtiter plates coated with A48 is only partially adsorbed by equal amounts of U10 and 3-76-42; (c) the binding of anti-A48 alkaline phosphatase-labeled antibody to U10 and 3-76-42 was completely adsorbed by A48 and only partially by 3-76-42. These results indicate that some of the A48 idiotopes are shared by U10, 3-76-42 and 3-76-36 monoclonal antibodies. It should be mentioned that 3-76-36 monoclonal antibodies probably share some idiotopes of U10 because the adsorption with U10 completely inhibited the binding of labeled anti-A48 to 3-76-36 coated plates, whereas the adsorption with

TABLE 5

Ability of Three β2→6 Fructosan-binding Monoclonal Antibodies to Adsorb the Binding Capacity of Anti-A48Id Antibodies

Microtiter plates coated with	Alkaline phosphatase-labeled anti-A48Id antibodies adsorbed with					
	Nil	M384	A48	U10	3-76-36	3-76-42
A48	1.85	1.86	0.05	1.15	ND	0.57
UPC10	0.55	0.53	0.01	0.12	ND	0.08
3-76-36	0.35	0.36	0.11	0.01	0.0	ND
3-76-42	0.39	0.38	0.02	0.05	ND	0.01

Microtiter plates were coated with 50 μg antibodies. Adsorption of labeled anti-A48Id antibodies (5 μg/ml) was performed by preincubation for 30 min at 23°C with 10 μg of affinity-purified monoclonal proteins. Optical density is at 405nm.

A48 caused only 60% inhibition of this binding. Based on these results, we investigated the ability of the three monoclonal antibodies that share A48 idiotopes to activate A48Id clones. The results presented in Table 6 show that treatment at birth with U10 and 3-76-42 followed by immunization with BL 1 month later elicited an anti-BL response whose magnitude was comparable with that of normal animals. The pretreatment with U10 and 3-76-42 caused a discrete activation of A48Id clones, whereas the pretreatment with 3-76-36 did not. This difference can be related to the fact that 3-76-36 monoclonal antibody shares only one or few A48 idiotopes that are not regulatory idiotopes as was assessed by partial inhibition of binding of labeled anti-A48 to 3-76-36 and subsequent absorption with A48 monoclonal protein (Table 5).

The inability of purified B cells to transfer the A48Id$^+$ response. The ability of B cells originating from animals that were treated at birth with A48 to confer A48 clonal dominance on the BL response in naive recipients was studied in transfer experiments. Purified B cells obtained from animals either injected with A48 at birth or not were prepared as described before (Materials and Methods). 5×10^7 B cells obtained from normal animals, A48-treated animals, or an equal mixture of both, were infused in lethally irradiated adult BALB/c mice (4-6 months old), which were subsequently immunized with BL. The anti-BL response was estimated by the PFC method 5 days after challenge. As can be seen in Table 7, no A48Id response was detected in these animals. These results clearly indicate that A48 treatment at birth does not directly stimulate A48Id B cell precursors, and that the B cells from A48-treated animals had no effect on B cells from the normal

TABLE 6

Treatment at Birth with Different Monoclonal Antibodies

Treatment at birth	Immunization at 1 mo	Antibody response		Idiotype response	
		BL	TNP	A48	M460
Nil	BL	5.0 ± 2.6	<3	<1	0.2 ± 0.2
Nil	TNP-Ficoll	0.7 ± 0.3	>100	2.5 ± 1.6	0.3 ± 0.0
A48	BL + TNP-Ficoll	37.1 ± 21.7	>100	173.3 ± 63.3	1.8 ± 0.8
U10	BL + TNP-Ficoll	1.1 ± 0.4	ND*	12.0 ± 6.4	1.8 ± 0.7
3-76-36	BL + TNP-Ficoll	2.0 ± 0.8	84.9 ± 39.2	<1	0.3 ± 0.1
3-76-42	BL + TNP-Ficoll	3.6 ± 1.8	37.0 ± 17.0	27.7 ± 5.0	0.9 ± 0.6

1-d-old BALB/c mice were injected with 10 µg monoclonal antibodies. 1-mo-old BALB/c mice were injected with 20 µg TNP-Ficoll. Antibody and idiotype concentrations were determined in RIA and expressed as mean ± SEM (µg/ml).

* Not determined.

TABLE 7

Failure to Transfer A48Id Response with B Cells from 1-mo-old Mice Treated at Birth with A48 Monoclonal Proteins

Lethally irradiated BALB/c mice infused with	Anti-BL PFC/spleen*	Number of mice studied	A48Id
			%
50×10^6 normal B cells	1,195 ± 225	2	0
50×10^6 A48 B cells	873 ± 335	4	0
25×10^6 normal B cells + 25×10^6 A48 B cells	850 ± 120	2	0

* Expressed as the mean ± SEM.

animals.

Expansion of A48Id-specific helper T cells. In further experiments, we investigated the effect of T cells from animals treated at birth with A48 on the activation of silent clones. In the first set of experiments, a mixture of 5×10^7 B cells plus 2.5×10^7 T cells from A48-treated animals was infused into lethally irradiated adult BALB/c mice, which were immunized with BL. An anti-BL PFC response comparable in magnitude with that of the animals infused with B cells alone was obtained. However, only the animals infused with B and T cells together developed an anti-BL PFC response, of which 90% expressed the A48Id (Table 8). An $A48Id^+$ response was not obtained in animals infused with B cells from A48-treated mice mixed with T cells from normal animals, or T cells from A48 mice treated with anti-Lyt-1.2 plus complement. The results of this experiment indicated that the expression of $A48Id^+$ BL-specific clones is dependent upon the presence of $Lyt\text{-}1.2^+$ helper cells that are lacking in the animals not treated at birth with A48. It should be mentioned that an infusion of equal numbers of T cells from normal animals together with A48-treated animals did not alter the A48Id response. These results show that the activity of these cells was not altered by putative, naturally occurring A48Id-specific suppressor cells (Lieberman et al., 1979), at least on a one-to-one cell basis ratio, as was assessed by the appearance of $A48Id^+$ cells in animals infused with a mixture of T cells purified from normal as well as mice treated with A48 at birth. To study the specificty of these cells we tried to enrich them on petri dishes coated with A48 monoclonal protein. Because we failed to enrich for or deplete whole splenic populations of these T cells by the

TABLE 8

Ability to Transfer A48Id$^+$ Response in Lethally Irradiated BALB/c Mice with Syngeneic T Cells from Mice Treated at Birth with A48 Monoclonal Protein

Mixture of cells infused into irradiated BALB/c mice					Anti-BL response	
B cells A48	T Cells				Total PFC/spleen	A48Id$^+$
	Normal	Treatment	A48	Treatment		
						%
Nil	Nil		Nil		10	0
50 x 10^6	Nil		Nil		873 ± 335	0
50 x 10^6	Nil		25 x 10^6	Nil	380 ± 10	91
50 x 10^6	Nil		25 x 10^6	Anti-Lyt-1.2 + C'[1]	140 ± 60	8
50 x 10^6	25 x 10^6	Nil	25 x 10^6	Nil	310 ± 10	91
50 x 10^6	25 x 10^6	Nil	25 x 10^6	Anti-Lyt-1.2 + C'	190 ± 40	24

B cells A48 and T cells A48 originate from 1-mo-old BALB/c mice injected at birth with 10 µg A48. Lethally irradiated mice after infusion of lymphocytes were injected intravenously with 20 µg BL and the anti-BL PFC response was studied 5 d later. The anti-BL PFC/spleen response is expressed as the mean ± SEM.

[1]C', complement.

plating method, we studied the specificity of these cells in nude BALB/c mice infused with T cells and immunized with an A48-TNP conjugate. We studied this response by measuring the anti-TNP PFC response. The rationale of this experiment was that if A48-treated mice generate A48Id-specific helper T cells, such cells will provide a helper effect to anti-TNP B cell precursors through an A48-TNP antigen bridge. Indeed, the infusion of purified T cells from mice treated with A48 at birth enabled nude BALB/c mice immunized with A48-TNP, but not with MOPC384 (M384)-TNP conjugates, to mount an anti-TNP response. The helper effect of these T cells was ablated by treatment with anti-Thy-1.2 and anti-Lyt-1.2 plus complement and was not altered by anti-Lyt-2.2 plus complement (Table 9). In another set of experiments aimed to determine the specificity of helper T cells, we studied the effects of treatment of these cells with A48 monoclonal protein and complement. The rationale of this experiment follows from previous findings in which it was shown that murine IgA-antigen complex can activate complement (Pfaffenbach et al., 1982) and that its binding to cellular antigen can cause the lysis of cells in presence of complement (Adinolfi et al., 1966). Kohler has succeeded in eliminating the T15Id-specific helper T cells by treatment with T15 and complement (personal communication). Therefore, T cells from BALB/c mice injected with 10 μg A48 at birth were incubated for 1 hour at 37°C with 1 mg A48 or M384 proteins and guinea pig complement diluted 1:3. After washing, 25×10^6 T cells were infused into four nude mice that were challenged with 50 μg TNP-A48 conjugate. Mice infused with T cells that were pretreated with M384 plus complement developed 3,538 $\pm$ 512 anti-TNP PFC/spleen, whereas the mice infused with T cells treated with A48 plus

TABLE 9

Demonstration of A48Id-specific Helper T Cells in BALB/c Mice Treated at Birth with 10 μg A48 Monoclonal Protein: Helper Effect on Anti-TNP Antibody Response in Nude Mice

Nude BALB/c mice infused with	Treatment of T cells	Immunization with with	Number of mice studied	Anti-TNP PFC/spleen
Nil		Nil	3	492 ± 79
Nil		TNP-Ficoll	2	90,200 ± 24,400
Nil		TNP-A48	2	275 ± 275
Nil		TNP-M384	2	378 ± 298
25 x 10^6 normal T cells		TNP-A48	4	490 ± 437
25 x 10^6 normal T cells		TNP-M384	4	212 ± 116
25 x 10^6 A48 T cells		TNP-A48	7	1,674 ± 653
25 x 10^6 A48 T cells		TNP-M384	2	5 ± 5
25 x 10^6 A48 T cells	Anti-Thy-1.2 + C'[1]	TNP-A48	2	50 ± 0
25 x 10^6 A48 T cells	Anti-Lyt-1.2 + C'	TNP-A48	6	524 ± 190
25 x 10^6 A48 T cells	Anti-Lyt-2.2 + C'	TNP-A48	4	935 ± 343

T cells were purified on nylon wool columns from spleens of 1-mo-old BALB/c mice treated at birth with 10 μg A48 monoclonal protein. The anti-TNP response was studied 7 d after infusion of T lymphocytes and immunization with TNP conjugates. All mice were immunized with 50 μg of the appropriate TNP conjugate. The anti-TNP PFC/spleen response is expressed as the mean ± SEM.

[1]C', complement.

complement showed a significant decrease in the anti-TNP PFC response (i.e., 883 $\pm$ 478). In this experiment, the control nude mice infused with 25 x 10^6 T cells from normal mice and subsequently challenged with TNP-A48 developed only 490 anti-TNP PFC/spleen. These results confirmed the presence of A48Id-specific T cells in mice treated at birth with A48 monoclonal protein.

Effect of injection of the UPC10 myeloma protein during pregnancy on the expression of U10Id$^+$ plaque forming cells in progeny. It has been an outstanding observation that the passive transfer of immunoglobulins to the fetus or newborn, by placental crossing or colostrum feeding, represents an important defense mechanism during the neonatal period, when the immune system is immature.

Previously discussed results clearly demonstrated that the parenteral administration of idiotypes at birth had a profound effect on the development of the repertoire after birth. In order to investigate the physiological significance of "idiotype driven" expansion of the repertoire, we have studied the effect of the injection of idiotypes during pregnancy on the expression of the corresponding idiotypes in the offspring. For this purpose we have chosen UPC10, and IgG2a myeloma protein instead of ABPC48, an IgA myeloma protein.

In preliminary experiments we have followed the placental transfer of radiolabelled UPC10 injected during pregnancy. In this experiment 10^7 cpm of ^{3}H-UPC10, representing 400 µg of protein, was injected into a pregnant BALB/c mouse. Fetuses were removed 24 hours later at which point radioactivity was measured in the fetal liver and spleen. A small amount of radioactivity corresponding to 8.0 ng of UPC10 in the

liver and 3.6 ng of UPC10 in the spleen was measured.

In further experiments we have studied the effect of injection of UPC10 during pregnancy on the U10Id$^+$ component of the anti-BL response. The results presented in Table 10 show that while the progeny from BALB/c mice injected during pregnancy with UPC10 developed a U10Id$^+$ response, those from mice injected with RPC5, and IgG2a myeloma protein with unknown binding specificity developed only a U10Id negative anti-BL response after immunization with 20 μg BL one month after birth.

Thus, these experiments clearly demonstrated that the maternal transfer of UPC10 into the fetus had a profound effect on the anti-BL repertoire. It has previously been discussed that the activation of A48Id$^+$ anti-BL clones after the administration at birth of A48Id bearing monoclonal antibodies is related to the expansion of A48Id specific helper T cells. The presence of such cells in this system was studied in adoptive transfer experiments in which B cells from TNP-Ova primed BALB/c mice (B-TNP) were infused into lethally irradiated BALB/c mice together with T cells from progeny originating from female mice injected during pregnancy with UPC10 (T-U10). These mice were injected with TNP-UPC10 or as controls TNP-RPC5 and TNP-MOPC384. MOPC384 is an IgA myeloma protein with α-methyl-D-galactoside binding specificity.

The data presented in Table 11 show that the mice reconstituted with B-TNP and T-U10 and immunized with TNP-UPC10 developed a higher anti-TNP PFC response compared to the mice infused with B-TNP only, B-TNP together with T-RPC5, B-TNP together with T-U10 and immunized with TNP-MOPC384 and B-TNP together with T-U10 and immunized with TNP-RPC5. The anti-TNP PFC response was only partially decreased after

TABLE 10

Effect of the Maternal Transfer of UPC10 on the Anti-BL Response in Progeny

Mice	Anti-BL PFC/spleen Total	%U10Id$^+$
Adult BALB/c	40,000 ± 15,558	9 ± 9
BALB/c females injected with UPC10	13,170 ± 4,628	30 ± 15
BALB/c progeny from females injected with UPC10	5,500 ± 1,622	93 ± 3
BALB/c females injected with RPC5	41,300 ± 15,000	7 ± 7
BALB/c progeny from females injected with RPC5	16,250 ± 4,534	5 ± 3

Females were injected i.v. with 100 µg UPC10 or RPC5 on a weekly basis during mating and throughout pregnancy. Injections ceased when progeny were born. The anti-BL plaque forming cell (PFC) response was measured 5 days after challenge with BL. Results are expressed as the mean ± SEM.

TABLE 11

Demonstration of U10Id Specific Helper T Cells in Progeny Originating from BALB/c Mice Injected During Pregnancy with UPC10 Myeloma Protein

Irradiated BALB/c mice infused with:		Immunized	
B Cells	T Cells	with	PFC/spleen
nil	nil	TNP-UPC10	70 ± 10
B-TNP	nil	TNP-UPC10	40 ± 0
B-TNP	T-RPC5	TNP-UPC10	70 ± 32
B-TNP	T-U10	TNP-MOPC384	65 ± 55
B-TNP	T-U10	TNP-RPC5	135 ± 85
B-TNP	T-U10	TNP-UPC10	200 ± 72
B-TNP	T-U10 + anti-Lyt1.2 + C'	TNP-UPC10	110 ± 52
B-TNP	T-U10 + anti-Lyt2.2 + C'	TNP-UPC10	663 ± 15

Mice were infused with equal numbers of both B cells and T cells (50×10^6). T cells were obtained from spleens by collecting the non-adherent cells from petri dishes coated with rabbit anti-mouse immunoglobulin serum. B cells were obtained by treating mice with rabbit anti-mouse thymocyte serum 2 days before removing the spleen and then treating the spleen cells with anti-Thy1.2 plus complement. All mice were immunized with 50 μg of the designated antigen. Anti-TNP PFC response was measured 5 days later and results are expressed as the mean ± SEM.

the pretreatment of T-U10 cells with anti-Lyt1.2 plus complement. Interestingly, a significant increase of the anti-TNP PFC response was observed when T-U10 cells were pretreated with anti-Lyt2.2 plus complement. These results suggest that the balance between clones which contribute to the BL response was profoundly altered and that both helper T cells and suppressor T cells had been activated. The ablation of suppressor T cells by anti-Lyt2.2 plus complement treatment led to a more prominant effect of U10Id$^+$ helper T cells. In speculating on the specificity of the receptor of the suppressor T cell, one could postulate that the receptor either be idiotype (U10) or anti-anti-U10Id (Ab3). It was shown in the A48 system that Ab3 and Ab1 actually share idiotopes and both bind Ab2. Thus, idiotype which crossed the placenta or was transferred in the colostrum to the neonate, could have interacted with the helper T cell and thereby overcome the effects of the suppressor T cell which, would account for the fact that U10Id$^+$ clones are normally not expressed in a conventional immune response with BL.

Genetic Control of Activation of A48Id Silent Clones After Manipulation of the Immune Network

Genetic control of activation of A48Id silent clones by anti-A48Id antibodies. In previous studies it was shown that either administration of minute amounts of anti-A48Id antibodies at birth (Hiernaux et al., 1981) or immunization of adult mice with anti-A48-KLH conjugates, (Bona et al., 1981) leads to the activation of A48Id silent clones. A significant increase of A48Id$^+$ anti-levan antibodies was not found in the sera of mice treated at birth with anti-M460Id antibodies. Therefore, the activation of A48Id silent clones was considered to be

an idiotype specific response. Furthermore, in mice treated with anti-A48Id antibodies and not immunized with BL, there was no activation of A48Id clones detected. Thus, this activation was not only idiotype specific, but required antigenic stimulation as well. Until now the expression of A48Id clones has only been studied in BALB/c mice, from which the ABPC48 myeloma originated. For this reason we have been interested in studying whether the expression of the A48Id$^+$ clones is linked only to IghV^a genes. In addition we investigated whether this response is under the control of MHC and/or IghC gene complexes.

In order to study the genetic control of the expression of A48Id, we studied this response in various strains of mice that differ in their MHC, IghC, and IghV haplotypes. These strains were treated at birth with anti-A48Id antibodies, followed one month later by immunization with bacterial levan. Only those mice treated with anti-A48Id, however, developed an A48Id$^+$ response. Activation of A48Id$^+$ silent clones was observed among all the strains. The results depicted in Table 12 indicate that all the mouse strains responded to immunization with BL, developing significant HA titers and a vigorous anti-levan PFC response.

A similar A48Id$^+$ anti-levan response was observed in adult mice after hyperimmunization with an anti-A48-KLH conjugate. Every strain of mice hyperimmunized with anti-A48-KLH conjugates produced significant HA titers of anti(anti-A48Id) antibodies varying from 2 to 5 $\log_2$ units. As expected, these mice developed significant HA anti-levan titers and a significant number of anti-levan PFC in response to immunization with bacterial levan. Furthermore, in these mice the

TABLE 12

Activation of A48Id Silent Clones by the Administration of 10ng Anti-A48Id Monoclonal Protein at Birth

Mouse Strain	Treatment	Antibody Response:Anti-BL HA[1]	Anti-BL PFC/Spleen[3]	A48Id$^+$ (Percent)	Idiotype Response:A48Id RIA[4]
BALB/c	nil	5.5 ± 0.3	7,833 ± 2,748	8 ± 4	<0.3
	Anti-A48Id	ND[2]	2,700 ± 460	52 ± 7	ND
BALB.B	nil	4.8 ± 1.1	24,067 ± 2,875	13 ± 7	0.7 ± 0.2
	Anti-A48Id	6.0 ± 0.4	4,683 ± 1,816	46 ± 15	20.7 ± 4.6
BALB.K	nil	7.0 ± 3.0	16,100 ± 3,300	ND	ND
	Anti-A48Id	6.3 ± 0.3	44,980 ± 8,985	51 ± 18	15.4 ± 3.5
BAB.14	nil	6.1 ± 0.4	18,975 ± 7,157	5 ± 5	1.4 ± 0.5
	Anti-A48Id	6.4 ± 0.5	34,650 ± 6,895	28 ± 9	36.9 ± 26.0
CCB.R4	nil	>8.0	4,125 ± 3,925	0 ± 0	ND
	Anti-A48Id	2.5 ± 0.9	3,488 ± 1,442	59 ± 17	21.6 ± 10.6
CAL.20	nil	3.4 ± 0.2	7,028 ± 3,225	ND	7.4 ± 3.5
	Anti-A48Id	3.5 ± 0.3	3,668 ± 944	ND	16.7 ± 5.8

[1]Mean log2 units ± SEM.

[2]Not determined.

[3]Mean ± SEM.

[4]RIA expressed as mean μg/ml ± SEM.

$A48Id^+$ clones were consistently activated subsequent to immunization with BL (Table 13). The adult mice that produce anti(anti-A48Id) antibodies, after hyperimmunization with anti-A48-KLH conjugates, were able to develop an $A48Id^+$ anti-levan response following immunization with bacterial levan.

The activation of A48Id silent clones in both experimental models was independent of the MHC gene complex. Indeed BALB/c, BALB.B, and BALB.K mice developed an $A48Id^+$ anti-levan response. In addition, activation was independent of the IghC gene complex. The ability of CAL.20 and BAB.14 mice to develop an $A48Id^+$ response clearly shows that activation is not associated with the IghC gene complex. Interestingly, both CAL.20 and CCB.R4 mice developed an $A48Id^+$ response, although they did not express $IghV^a$ genes.

Our results taken collectively indicate that A48Id silent clones can be activated by administration at birth of anti-A48Id monoclonal antibodies or during adult life by hyperimmunization with anti-A48-KLH conjugates. This activation occurs independently of the MHC, IghC, and IghV gene complexes.

Genetic control of activation of A48Id silent clones by administration at birth of A48Id monoclonal antibodies. The previous data has shown that BALB/c mice injected at birth with A48Id monoclonal antibodies led to the activation and dominance of the $A48Id^+$ component of an anti-levan response. The present data suggests that the activation of $A48Id^+$ clones occurs independently of MHC, IghC, and $IghV^a$ gene complexes. The data show that BALB/c, BALB.B, BALB.K, BAB.14, CAL.20, and CCB.R4 mice were all able to develop an $A48Id^+$ anti-levan response following treatment at birth with A48Id monoclonal

TABLE 13

Activation of A48Id Silent Clones by Hyperimmunization of Adult Mice with Anti-A48-Keyhole Limpet Hemocyanin

Mouse Strain	Treatment	Antibody Response:Anti-BL HA[1]	Anti-BL PFC/Spleen[2]	A48Id$^+$ (Percent)	Idiotype Response:A48Id RIA[4]
BALB/c	nil	5.5 ± 0.3	7,833 ± 2,748	8 ± 4	<0.3
	Anti-A48-KLH	7.8 ± 3.2	765 ± 433	32 ± 20	4.8 ± 1.6
BALB.B	nil	4.8 ± 1.1	24,067 ± 2,875	13 ± 7	0.7 ± 0.2
	Anti-A48-KLH	>8.0	183,400 ± 45,061	24 ± 3	36.0 ± 10.0
BALB.K	nil	7.0 ± 3.0	16,100 ± 3,300	ND[3]	ND
	Anti-A48-KLH	6.5 ± 0.5	85,700 ± 5,300	ND	20.1 ± 11.4
BAB.14	nil	6.1 ± 0.4	18,975 ± 7,157	5 ± 5	1.4 ± 0.5
	Anti-A48-KLH	8.0 ± 0.6	69,550 ± 14,663	11 ± 6	27.4 ± 12.2
CCB.R4	nil	>8.0	4,125 ± 3,925	0 ± 0	ND
	Anti-A48-KLH	6.0 ± 0.6	36,933 ± 18,859	12 ± 7	46.6 ± 23.2
CAL.20	nil	3.4 ± 0.2	7,028 ± 3,225	ND	7.4 ± 3.5
	Anti-A48-KLH	5.7 ± 0.3	91,667 ± 24,891	29 ± 14	23.0 ± 15.1

[1]Mean log2 units ± SEM.

[2]Mean ± SEM.

[3]Not determined.

[4]RIA expressed as mean μg/ml ± SEM.

antibody and immunization one month later with BL (Table 14).

Genetic control of activation of U10Id silent clones by in utero exposure to U10Id monoclonal antibodies. The results show that BALB/c, BALB.B, and BAB.14 mice developed a U10Id$^+$ response, whereas CCB.R4 did not (Table 15). These preliminary results clearly indicate that the activation of U10Id silent clones subsequent to in utero exposure to homologous protein led to an idiotype-induced-idiotype response, similar to that induced by parenteral administration of idiotypes after birth. This idiotype-induced-idiotype response is independent of MHC and IghC gene complexes, but is IghV^a restricted. The IghV^a restriction of this response can be related either to the nature of U10Id or to the specificity of U10Id T-helper cells, which can be IghV restricted.

The Requirement for Immunization with Antigen or Monoclonal Anti-idiotypic Antibodies for the Activation of β2→6 and β2→1 Polyfructosan-reactive Clones in BALB/c Mice Treated at Birth with Minute Amounts of Anti-A48 Idiotype Antibodies

Requirement of antigenic challenge for the activation of the A48Id$^+$ response in BALB/c mice treated at birth with anti-A48Id antibodies. The A48Id of a β2→6 fructosan-binding myeloma protein. ABPC48, from the BALB/c mouse is not expressed on antibodies of various mouse strains during a conventional immune response after immunization with BL. However, the study of the anti-β2→6 fructosan response in 1-month-old BALB/c mice treated at birth with 0.01-10 μg of anti-A48Id antibodies demonstrated the appearance of A48Id$^+$ anti-β2→6 fructosan PFC (Fig.3). The percentage of A48Id$^+$ PFC was significantly higher in mice treated at birth with anti-A48Id antibodies than in mice not so

TABLE 14

Activation of A48Id Silent Clones by the Administration of 10 μg of A48Id Monoclonal Protein at Birth

Mouse Strain	Treatment	Antibody Response:Anti-BL HA[1]	Anti-BL PFC/Spleen[2]	A48Id$^+$ (Percent)	Idiotype Response:A48Id RIA[4]
BALB/c	nil	5.5 ± 0.3	7,833 ± 2,748	8 ± 4	<0.3
	A48Id	5.7 ± 0.6	21,133 ± 2,245	95 ± 1	82.4 ± 47.6
BALB.B	nil	4.8 ± 1.1	24,067 ± 2,875	13 ± 7	0.7 ± 0.2
	A48Id	4.1 ± 0.7	869 ± 298	55 ± 14	32.0 ± 14.6
BALB.K	nil	7.0 ± 3.0	16,100 ± 3,300	ND[3]	ND
	A48Id	3.0 ± 1.1	ND	ND	18.3 ± 9.0
BAB.14	nil	6.1 ± 0.4	18,975 ± 7,157	5 ± 5	1.4 ± 0.5
	A48Id	3.7 ± 1.2	4,511 ± 1,223	47 ± 12	15.2 ± 4.2
CCB.R4	nil	>8.0	4,125 ± 3,925	0 ± 0	ND
	A48Id	4.6 ± 0.5	1,811 ± 629	26 ± 8	27.1 ± 10.4
CAL.20	nil	3.4 ± 0.2	7,028 ± 3,225	ND	7.4 ± 3.5
	A48Id	4.5 ± 0.5	1,375 ± 125	ND	63.0 ± 22.5

[1]Mean log2 units ± SEM.

[2]Mean ± SEM.

[3]Not determined

[4]RIA expressed as mean μg/ml ± SEM.

TABLE 15

Activation of U10Id Silent Clones by In Utero Exposure to U10Id Monoclonal Protein

Mouse Strain	Treatment	Anti-BL PFC/Spleen[1]	U10Id$^+$ (Percent)
BALB/c	nil	40,000 ± 15,558	9 ± 9
	U10 females	13,170 ± 4,628	30 ± 15
	offspring	5,500 ± 1,622	93 ± 3
BALB.B	nil	60,600 ± 13,500	10 ± 9
	U10 females	116,000	14
	offspring	16,950 ± 14,203	43 ± 28
BAB.14	nil	15,867 ± 9,558	0
	U10 females	34,575 ± 15,225	14 ± 14
	offspring	5,450 ± 1,931	42 ± 11
CCB.R4	nil	4,125 ± 3,925	2 ± 2
	U10 females	30,833 ± 7,946	3 ± 3
	offspring	7,392 ± 2,333	5 ± 2

[1]PFC expressed as mean ± SEM.

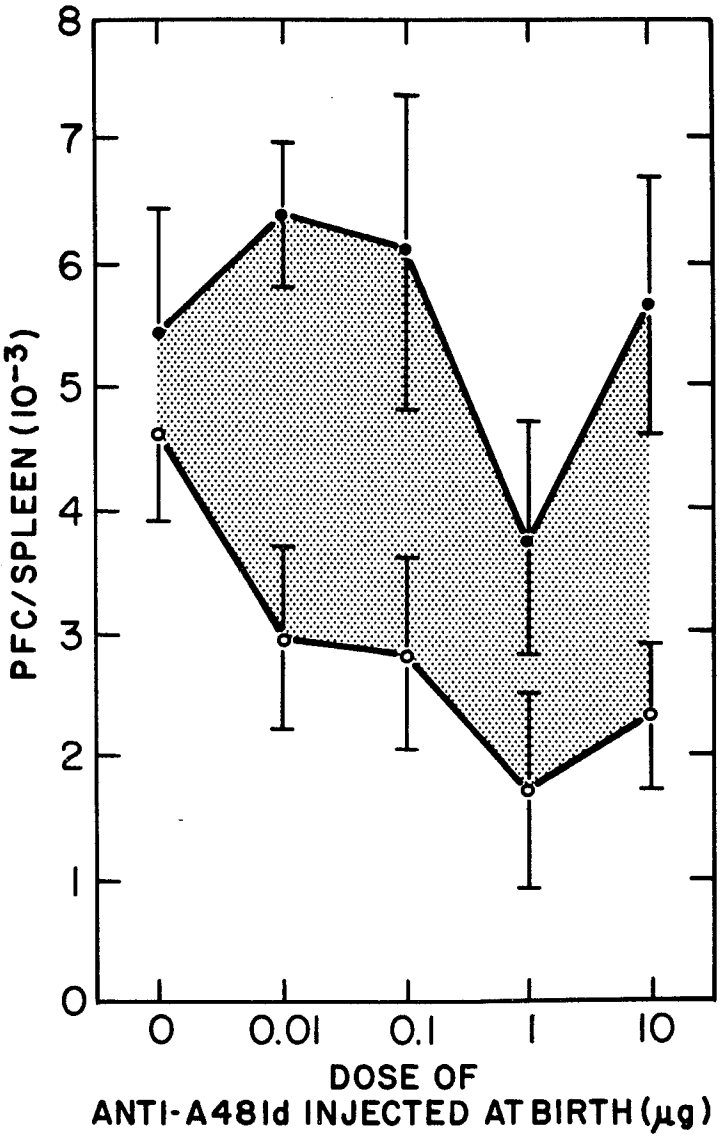

FIGURE 3. Dose-effect relationship of treatment of newborn mice with various doses of anti-A48Id polyclonal antibody. Mice were 1-day-old at the time of treatment and challenged 1 month later with 20 μg BL. (●) Anti-BL PFC/spleen; (○) anti-BL PFC/spleen detected when syngeneic anti-A48Id antisera had been added to the agarose. Shaded area represents the number of PFC bearing the A48Id. Each point represents the mean ± SEM of determinations performed on five mice.

treated. The immunization with BL was critical for the activation, since mice treated at birth with anti-A48Id antibodies and not immunized with BL did not develop a β2→6 fructosan antibody response. Therefore, it appears that the administration at birth of anti-A48Id antibodies was not sufficient by itself to activate A48Id$^+$ anti-β2→6 fructosan-specific clones, but must be supplemented with antigenic challenge. It was previously demonstrated (Hiernaux et al., 1981) that this activation is idiotype-specific since the treatment with anti-A48Id, but not the treatment with anti-M384Id, followed by immunization with BL 1 month later, led to the activation of A48Id$^+$ clones. The M384Id is borne on the MOPC384 myeloma protein that binds α-methyl-D-galactoside, the immunodominant sugar shared on the lipopolysaccharide of Salmonella tranaroa, Salmonella telaviv, and Proteus mirabilis (Hiernaux and Bona, 1982). Furthermore, BALB/c mice treated at birth with anti-A48Id antibody and immunized 1 month later with TNP-Ficoll did not show a significant increase of the A48Id$^+$ component of the anti-β2→6 fructosan response, nor of the M460Id$^+$ component of the anti-TNP response (Table 16). Therefore, the effect produced by anti-A48Id$^+$ antibodies is specific, since treatment with anti-A48Id did not alter the M460Id$^+$ component of the anti-TNP response.

Long-lasting activation of precursors of A48Id$^+$ antibody-producing cells after treatment at birth with anti-A48Id antibodies. The persistence of activation of A48Id$^+$ bearing clones after treatment of mice at birth with 0.01 and 10 μg of anti-A48Id antibodies was studied in BALB/c mice immunized at various ages. In these experiments the BALB/c mice after treatment at birth were immunized with 20 μg of BL at either 4, 6, 8, or 12 weeks and their anti-BL PFC response was measured

TABLE 16

Requirement of Antigen for the Activation of the A48Id$^+$ Response in BALB/c Mice Treated at Birth with Anti-A48Id Antibody

Mice treated at birth with:	Mice challenged with:	Anti-BL PFC/spleen[1]		Anti-TNP PFC/spleen[2]	
		Total	A48Id$^+$	Total	M460Id$^+$
			%		%
Nil	20 µg BL	2,180 ± 513	6 ± 3	<100	ND[3]
Nil	20 µg TNP-Ficoll	<100	ND	44,800 ± 3,582	16 ± 7
0.01 µg anti-A48Id	Nil	<100	ND	<100	ND
0.01 µg anti-A48Id	20 µg BL	2,920 ± 718	52 ± 7	<100	ND
0.01 µg anti-A48Id	20 µg TNP-Ficoll	<100	ND	39,700 ± 7,860	24 ± 6

[1]Total anti-BL PFC/spleen response was measured 5 d after immunization. The percentage of A48Id$^+$ anti-BL PFC was determined by the addition of anti-A48Id antisera to the agarose and scoring the difference in the number of PFC without antisera. Results represent the mean ± SEM for the data obtained from five mice.
[2]Total anti-TNP PFC/spleen response was measured 5 d after immunization. The percentage of M460Id$^+$ anti-TNP PFC was determined by the addition of anti-M460Id antisera to the agarose and scoring the difference in the number of PFC without antisera. Results represent the mean ± SEM for the data obtained from five mice.
[3]Not determined, insufficient number of plaques.

5 days later. The data depicted in Table 17 shows that the A48Id$^+$ component represents an insignificant fraction varying between 6 and 18% of the anti-BL response in 4-12-week-old BALB/c mice. The A48Id$^+$ component significantly increases (35-79%) in mice treated at birth with 0.01 or 10 μg of anti-A48Id antibodies and challenged with the antigen, BL. Although the W3082IdX anti-β2→6 and β2-1 fructosan-reactive clones become dominant in 6-week-old mice (Bona et al., 1979) an A48Id$^+$ anti-β2→6 fructosan response was still observed in 6-12-week-old mice treated at birth with anti-A48Id antibodies. Thus, the administration at birth of anti-A48Id antibodies has a profound effect on B cell precursors because it causes a long-lasting activation during postnatal life.

A monoclonal anti-A48Id antibody is able to replace the antigenic challenge required for the activation of an anti-β2→6 fructosan response. The previous experiments demonstrated that antigenic challenge with BL was critical for the differentiation of A48Id$^+$ anti-β2→6 fructosan precursors. In a pilot experiment we screened several syngeneic monoclonal anti-A48Id antibodies for their ability to elicit an anti-β2→6 fructosan PFC response in mice treated at birth with polyclonal anti-A48Id. These monoclonal antibodies recognize shared idiotopes on A48 and U10 monoclonal proteins (Goldberg et al., 1983). The results depicted in Table 18 show that the injection of 10 μg of 17-38 monoclonal antibody instead of BL 1 month after the treatment at birth with polyclonal anti-A48Id antibodies induced a significant anti-BL PFC response. Monoclonal anti-A48Id antibodies, including 17-38, did not inhibit any particular property with respect to their ability to bind labeled A48 or anti-(anti-A48Id) antibodies.

TABLE 17

Long-lasting Activation of the A48Id$^+$ Response After Treatment at Birth with Anti-A48Id Antibody

Age of mice when immunized with 20 µg BL	PFC/spleen after treatment at birth with[1]					
	Nil		0.01 µg anti-A48Id		10 µg anti-A48Id	
	Total	A48Id$^+$	Total	A48Id$^+$	Total	A48Id$^+$
wk		%		%		%
4	2,180 ± 513	6 ± 3	2,920 ± 718	52 ± 7	688 ± 85	39 ± 13
6	1,300 ± 120	14 ± 2	675 ± 196	79 ± 10	2,387 ± 950	53 ± 9
8	6,270 ± 480	18 ± 9	3,362 ± 2,234	46 ± 13	6,175 ± 1,561	49 ± 5
12	13,140 ± 5,125	11 ± 3	9,150 ± 2,850	47 ± 12	13,490 ± 3,054	35 ± 8

[1]Mean ± SEM for PFC/spleen detected for five mice, 5 d after immunization.

TABLE 18

Ability of 17-38 Monoclonal Anti-A48Id Antibody to Elicit an Anti-BL PFC Response in Mice Treated at Birth with 0.01 μg of Anti-A48 Id Antibody

Monoclonal anti-A48Id antibody*	Isotype	Anti-BL PFC/spleen
26-125	IgG1	180
148-3	IgG1	140
100-105	IgG2b	180
23-113	IgM	120
14-114	IgM	40
17-38	IgM	2,120

* Each mouse received a 10 μg injection of the appropriate monoclonal anti-A48Id antibody and the anti-BL PFC response was measured 5 d later. Results are expressed as the mean for two mice.

In addition, the binding of labeled A48 to these monoclonal Ab2 was inhibited by BL (0.01-0.1 μg/ml) (data not shown). This "antigen-like" effect was obtained with various concentrations (1, 10, 100 μg) of 17-38 monoclonal antibodies (Fig.4). In further experiments we studied the activation of both β2→6 and β2→1 fructosan-reactive clones in mice treated at birth with anti-A48Id antibodies or BL and challenged 1 and 3 months later with 1 μg of 17-38 monoclonal antibody. The rationale of this experiment followed our previous observation, that there was a substantial ontogenic delay of the anti-β2→1 fructosan antibody response. The results depicted in Table 19 show that BALB/c mice treated at birth with anti-A48Id antibodies or BL and challenged 1 month later with 17-38 monoclonal antibody develop only an A48Id negative anti-BL response. It should be noted that only a U10Id$^+$ anti-BL response was observed in mice treated at birth with BL and injected 1 month later with 17-38. By contrast, in mice challenged with BL, 50% of the anti-β2→6 fructosan PFC expressed the A48Id. Furthermore, the mice treated at birth with anti-A48Id antibodies and challenged 3 months later with 17-38 monoclonal antibody developed an anti-β2→6 and anti-β2 1 fructosan response expressing the dominant W3082 IdX. W3082 monoclonal protein shares IdX G, B, and A of E109 and J606 In-binding monoclonal proteins (Lieberman et al., 1975). One month and 3 month old BALB/c mice not treated at birth with anti-A48Id antibodies and injected with 1 μg 17-38 monoclonal antibody did not develop an anti-β2→6 or anti-β2→1 fructosan PFC response (data not shown). In addition, in both groups of mice the anti-TNP PFC response and M460Id$^+$ component were not augmented in the mice treated at birth with anti-A48Id antibodies and challenged 1 or 3 months later with

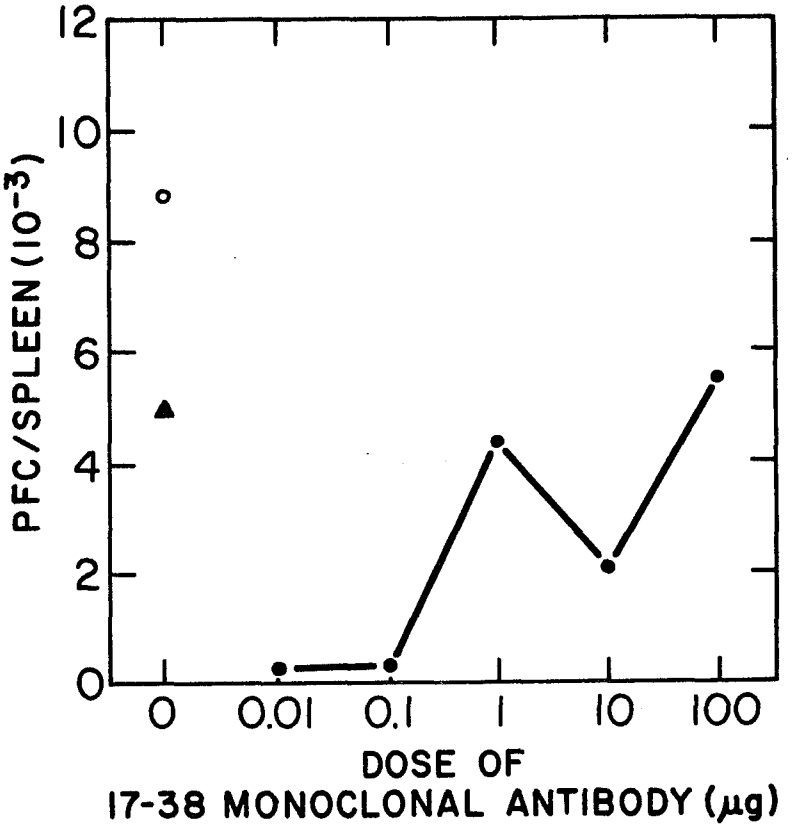

FIGURE 4. Dose-effect relationship of the challenge with various doses of 17-38 monoclonal anti-A48Id antibodies. Mice were 1-day-old at the time of treatment with 10 ng polyclonal anti-A48Id antibody and were challenged 1 month later with various doses of 17-38 monoclonal anti-A48Id antibody. (●) Anti-BL PFC/spleen; (○) anti-BL PFC/spleen of mice treated at birth with anti-A48Id antibody and challenged 1 month later with 20 μg BL; (▲) anti-BL PFC/spleen of normal mice challenged with 20 μg BL at 1 month of age. Each point represents the mean of determinations performed on two mice.

TABLE 19

Age Dependence and the Specificity of Activation of Anti-β 2→6 and Anti-β 2→1 Fructosan Clones in BALB/c Mice Treated at Birth with 0.01 μg Anti-A48Id Antibody and Challenged with 17-38 Monoclonal Antibody

Mice treated at birth with:	Challenged with:[1]	Age of mice	Anti-BL PFC/spleen[2]				Anti-In PFC/spleen		Anti-TNP PFC/spleen	
			Total	A48Id$^+$	U10Id$^+$	W3082 IdX$^+$	Total	W3082 IdX$^+$	Total	M460 Id$^+$
		mo		%	%	%		%		%
Nil	BL	1	2,850 ± 824	14 ± 7	29 ± 16	76 ± 9	<100	ND[3]	<100	ND
Nil	TNP-Ficoll	1	<100	ND	ND	ND	<100	ND	48,600 ± 6,713	1 ± 1
BL[4]	Nil	1	<100	ND	ND	ND	<100	ND	<100	ND
Anti-A48Id	Nil	1	<100	ND	ND	ND	<100	ND	<100	ND
BL	BL	1	4,100 ± 2,800	5 ± 5	14 ± 14	23 ± 23	<100	ND	<100	ND
Anti-A48Id	BL	1	2,920 ± 718	52 ± 7	NP	NP[5]	<100	ND	<100	ND
BL	17-38	1	1,750 ± 310	0	33 ± 1	30 ± 3	310 ± 70	0	<100	ND
Anti-A48Id	17-38	1	1,815 ± 225	7 ± 7	8 ± 6	49 ± 13	<100	ND	<100	ND
Nil	BL	3	15,417 ± 5,140	15 ± 10	34 ± 6	57 ± 24	3,400 ± 1,588	85 ± 9	<100	ND
Nil	TNP-Ficoll	3	<100	ND	ND	ND	<100	ND	97,300 ± 18,631	0
Anti-A48Id	Nil	3	<100	ND	ND	ND	<100	ND	<100	ND
Anti-A48Id	BL	3	9,150 ± 2,850	47 ± 12	NP	NP	NP	NP	<100	ND
Anti-A48Id	17-38	3	6,750 ± 3,384	0	14 ± 7	78 ± 9	5,250 ± 2,905	76 ± 5	925 ± 364	28 ± 24

[1]Mice were challenged with either 20 μg BL, 20 μg TNP-Ficoll, or 1 μg 17-38.
[2]All PFC results are expressed as the mean ± SEM. The percentage of Id+ PFC in each group were detected by plaque inhibition experiments incorporating the appropriate antisera and scoring the difference in the number of plaques vs. the total.
[3]Not determined, insufficient number of plaques.
[4]Mice were immunized at birth with 10 μg BL.
[5]Experiment not performed.

17-38 monoclonal antibodies.

Analysis of IgG antibodies by IEF. Sera from 1-and 3-month-old mice treated or not at birth with anti-A48Id antibodies and challenged with BL or 17-38 were analyzed by IEF with ^{125}I-In-BSA and ^{125}I-BL. In the sera of three individual 1-month-old BALB/c mice immunized with BL, a set of faint bands that bound BL and focused between pH 7 and 8.2 were observed (Fig.5). The dark curved bands seen in this group and in two of the three sera in the anti-A48 plus-BL group that focus between pH 6.8 and 7.2, are nonspecific and are due to the fact that these sera were highly hemolyzed. The bands focusing between pH 7 and 8.2 correspond to the major anti-BL specrotype described previously in adult BALB/c mice (Stein et al., 1980). An additional spectrotype, also described, can be seen in serum 1 in this group. These spectrotypes were not observed in sera of mice treated at birth with anti-A48Id antibodies and immunized 1 month later with BL. Some BL binding was seen, however, in the region between pH 5.7 and 6.2. These antibodies and U10, which also focused in this region and was poorly resolved, were specific for BL. All the mice treated at birth with anti-A48Id antibodies and challenged 1 month later with 17-38 produced an IgG anti-BL response consisting of five spectrotypes focusing between pH 7 and 8.5 (Fig.5). Again, these spectrotypes were the same as those previously shown in the sera of adult BALB/c mice immunized with BL. No IgG anti-In antibodies were detected in the sera of 1-month-old mice in all three groups (data not shown). The sera of 3-month-old normal mice immunized with BL showed the characteristic BALB/c anti-In spectrotype shown previously (Stein et al., 1980) (Fig.6). This spectrotype has the same focusing pattern as J606 (i.e.,

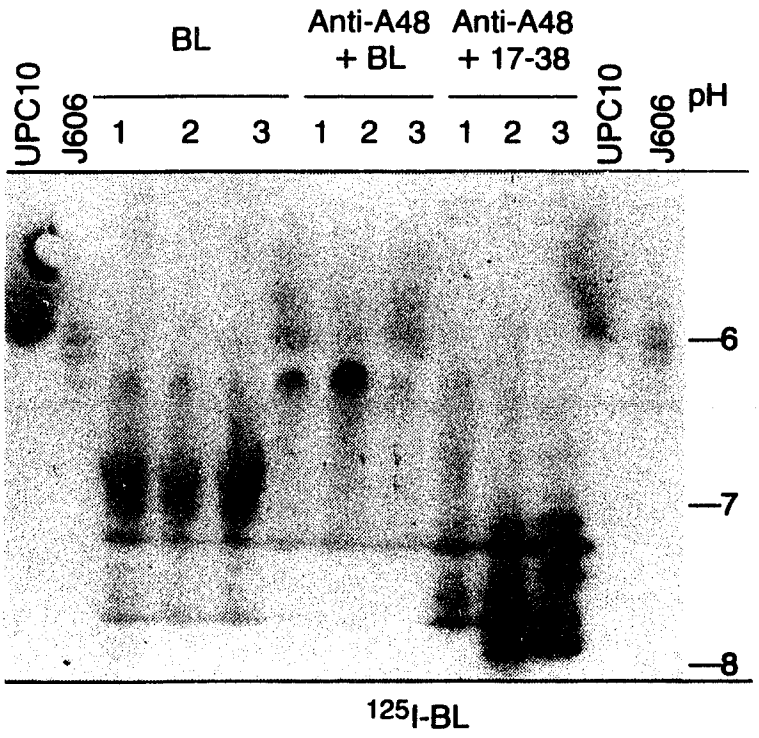

FIGURE 5. 10 µl of serum from individual, 1-month-old BALB/c mice were analyzed by IEF autoradiography using ^{125}I-BL antigen overlay. The groups are indicated above the autoradiogram and the individual sera are indicated by the numbers within each group. 10 µl of a 1 mg/ml solution of U10 and J606 purified myeloma proteins were applied to the gel for reference. Autoradiograms were obtained by exposing the film for 8-10 day.

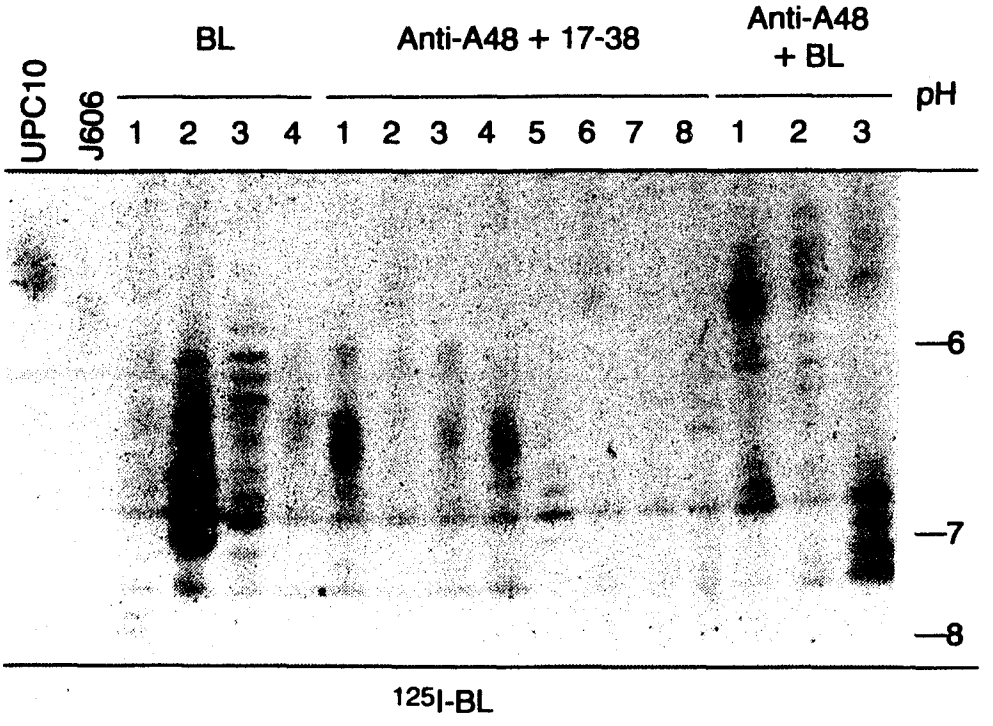

FIGURE 6. 10 µl of serum from individual, 3-month-old BALB/c mice were analyzed by IEF autoradiography using a ^{125}I-BL antigen overlay. The groups are indicated above the autoradiogram and the individual sera are indicated by the numbers within each group. 10 µl of a 1 mg/ml solution of U10 and J606 purified myeloma proteins were applied to the gel for reference. Autoradiograms were obtained by exposing the film for 8-10 day.

an In-binding myeloma protein of the IgG3 subclass). This spectrotype was also observed (Fig.6) in three mice treated at birth with anti-A48Id antibodies and immunized 3 months later with BL. However, BL binding showed that this spectrotype in the sera from anti-A48Id-treated mice bound better to BL than to In (Fig.7).

Stein et al. (1980) previously reported that the recombinant inbred strain, CXBJ, also has the characteristic BALB/c anti-In spectrotype. We report here that this spectrotype in both CXBJ and BALB/c sera is indistinguishable from that of the J606 myeloma (Fig.8). We have observed, however, that late in the response after a single immunization with BL, this spectrotype shows preferential binding to BL rather than In in sera from CXBJ mice (Fig.9), but not in sera from BALB/c mice (Stein et al. 1980). Lieberman et al. (1979) reported that adult CXBJ mice, but not adult BALB/c mice, after treatment with anti-E109 (anti-IdX) and immunization with BL, express A48 anti-BL. These data taken together suggest that the BALB/c anti-In spectrotype is comprised of two antibody clones, one of which preferentially binds BL rather than In and which may bear the A48Id. In the sera of BALB/c mice treated at birth with anti-A48Id and immunized at 3 months of age with BL, we observed anti-BL antibody spectrotypes characteristic of normal BALB/c mice (Fig.7). The sera of mice treated at birth with anti-A48Id antibodies and challenged 3 months later with 17-38, showed typical BALB/c In and BL spectrotypes when present. Therefore, the PFC and IEF data showed important alterations in the expression of anti-BL and anti-In clones in animals treated at birth with anti-A48Id antibodies and immunized later with BL or 17-38.

T independence of the activation of A48Id$^+$ clones after treatment

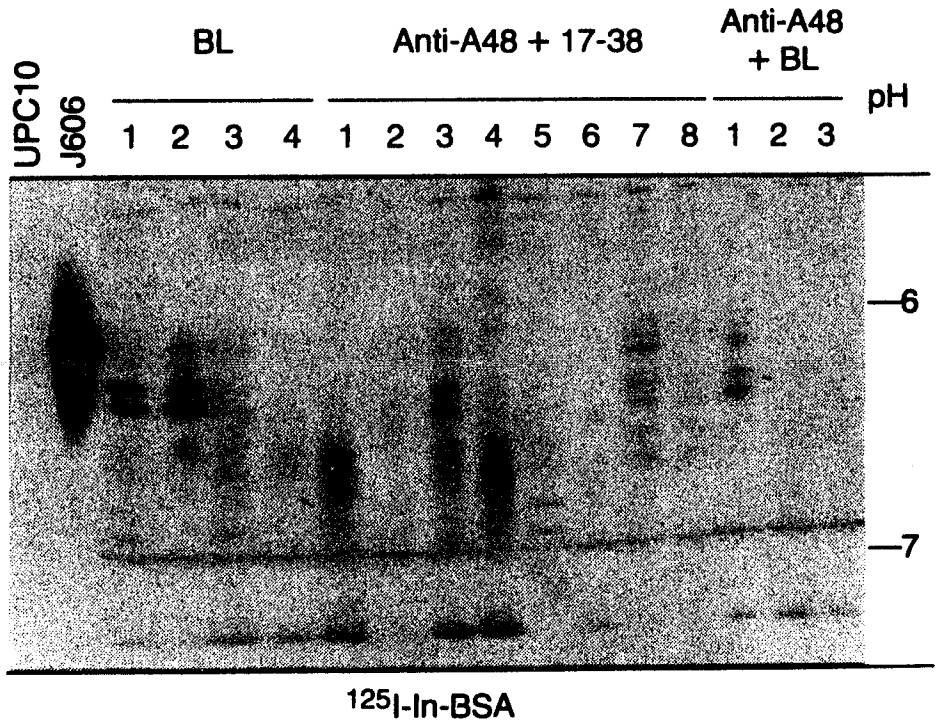

FIGURE 7. 10 µl of the same sera from the individual, 3-month-old BALB/c mice, as were shown in Fig. 6, were analyzed using a ^{125}I-In-BSA overlay. The groups are indicated above the autoradiogram and the individual sera are indicated by the numbers within each group. 10 µl of a 1 mg/ml solution of U10 and J606 purified myeloma proteins were applied to the tel for reference. Autoradiograms were obtained by exposing the film for 8-10 day.

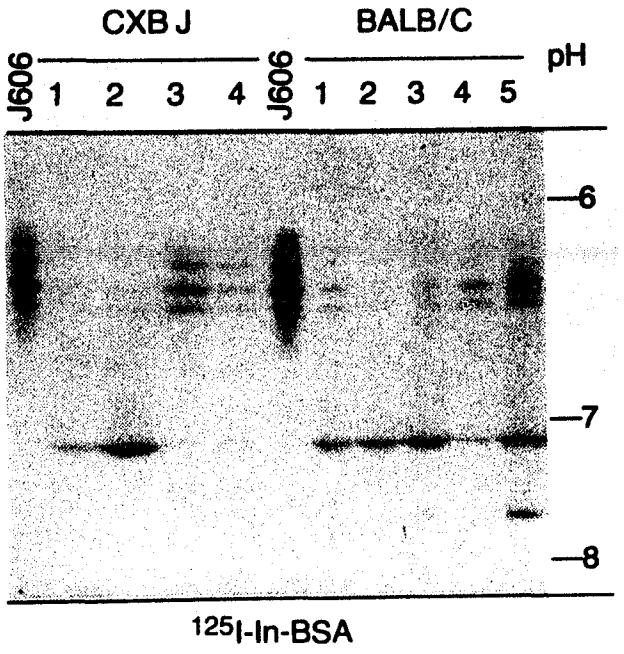

FIGURE 8. 10 µl of serum obtained from individual CXBJ and BALB/c mice 10 day after intravenous immunization with 20 µg BL were focused and overlaid with ^{125}I-In-BSA. 10 µl of a 1 mg/ml solution of J606 were applied for reference.

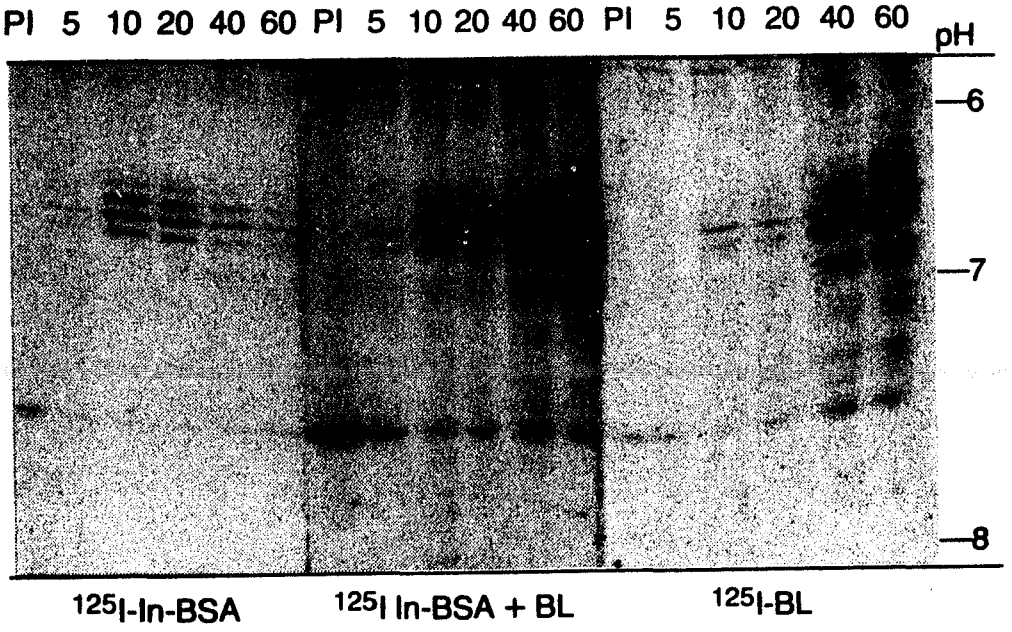

FIGURE 9. 10 µl of serum from an individual CXBJ mouse obtained before immunization or 5, 10, 20, 40 and 60 days after intravenous immunization with 20 µg BL, were focused in triplicate and each of the three panels was overlaid with radioactive antigen as indicated.

at birth with anti-A48Id antibodies. In further experiments we investigated the requirement of T cells for the $A48Id^+$ response. Thus, highly purified T and B cells from normal mice and mice treated at birth with anti-A48Id antibodies were infused alone or in different combinations into lethally irradiated BALB/c mice. The mice were immunized with 20 μg BL at the time of cell transfer and the anti-BL PFC response was measured 5 days later. The data depicted in Table 20 show that the transfer of B cells from 1-month-old animals treated at birth with anti-A48Id antibodies into lethally irradiated BALB/c mice, followed by immunization with BL, was sufficient to obtain an $A48Id^+$ PFC response. The infusion of B cells together with T cells from either normal animals or animals treated at birth with anti-A48Id antibodies into lethally irradiated BALB/c mice, did not significantly alter the expression of $A48Id^+$ clones. Furthermore, the infusion of B cells from normal animals along with T cells from animals treated at birth with anti-A48 Id antibodies, did not elicit an $A48Id^+$ response. Therefore, these results suggest that there is a direct interaction of anti-A48Id antibodies with the immunoglobulin receptor of $A48Id^+$ B cell precursors, which is responsible for their activation.

Regulation of Myeloma Growth by Antigen and Regulatory Idiotopes

Kinetics of myeloma growth. After the adaptation of the ascitic form of ABPC48 myeloma cells to home directly to and grow in splenic tissue when injected i.v. into mice, the titration and subsequent determination of the amount of myeloma cells which allowed the animals to survive 14 to 28 days was analyzed. As shown in table 21, mice injected with 1×10^3 myeloma cells survived 28 days, but a time dependent growth of the myeloma was observed as estimated by three of

TABLE 20

Transfer of A48Id$^+$ Response with B Cells from Mice Treated at Birth with 0.01 μg of Anti-A48Id Antibody

Lethally irradiated BALB/c mice infused with:		Number of mice	Anti-BL PFC/spleen[3]	
B cells[1]	T cells[2]		Total	A48Id$^+$
				%
Nil	Nil	2	180 ± 170	7 ± 7
Normal	Nil	2	843 ± 375	0
Anti-A48	Nil	7	1,857 ± 964	46 ± 14
Normal	Anti-A48	3	940 ± 530	0
Anti-A48	Normal	7	1,094 ± 449	42 ± 11
Anti-A48	Anti-A48	2	650 ± 75	28 ± 6

[1] 40×10^6 B cells isolated from either normal BALB/c mice or BALB/c mice treated at birth with 0.01 μg anti-A48Id.

[2] 20×10^6 T cells isolated from either normal BALB/c or BALB/c mice treated at birth with 0.01 μg anti-A48Id.

[3] Anti-BL PFC response was assayed 5 d after the infusion of B and/or T cells along with 20 μg BL. The percentage of A48Id+ PFC was determined by adding anti-A48Id antisera into the agarose and scoring the difference in the number of plaques vs. the total response. Results are expressed as the mean ± SEM for the number of mice indicated.

TABLE 21

Relationship between the number of myeloma cells injected and the growth of the myeloma

Number of myeloma cells injected i.v.	Day 14			Day 21			Day 28		
	Spl-Wt[1]	[^{3}H]Td[2]	IgA[3]	Spl-Wt	[^{3}H]Td	IgA	Spl-Wt	[^{3}H]Td	IgA
1 x 10^3	0.15 ± 0.01	1.0 ± 0.3	<10	0.43 ± 0.14	2.2 ± 1.0	<10	0.56 ± 0.11	11.8 ± 3.2	220 ± 12
1 x 10^4	0.72 ± 0.12	3.3 ± 0.8	88 ± 22	0.80 ± 0.08	11.8 ± 1.7	98 ± 5		DIED	
1 x 10^5	0.71 ± 0.05	7.0 ± 0.5	>1000		DIED			----	

[1]Spleen weight (Spl-Wt) is expressed as the mean (g) ± SE.

[2]Relative proliferation of 2.5 x 10^5 spleen cells incubated for 4 hours at 37°C in RPMI 1640 media supplemented with glutamine and antibiotics, and the addition of 0.1 µCi [^{3}H] Thymidine ([^{3}H]Td). Results are expressed as mean (cpm x 10^{-3}) ± SE.

[3]Titer of anti-levan IgA in the sera as determined from an RIA. Results are expressed as the mean (µg/ml) ± SE.

the criteria used to measure the development of the myeloma, namely survival, spleen weight, and proliferation of tumor cells which homed into and grew in the spleen as assessed by measuring the in vitro incorporation of [^{3}H]thymidine.

Effects of antigen immunization on the growth of ABPC48 myeloma cells. Previous findings have shown that the in vitro proliferation of MOPC-104E, TEPC15 or MOPC315 myeloma cells are inhibited by α,1-3 dextran, phosphorylcholine or DNP-conjugates respectively (Bhoopalam et al, 1980; Paraf et al., 1975; Abbas and Klaus, 1977). Two methods were used to explore the role of antigen in vivo on the growth and development of ABPC48 myeloma cells. Mice were immunized with either immunogenic or tolerogenic doses of antigen. Preliminary experiments investigated various schedules of immunization and myeloma transplantation had on tumor growth. In the first schedule mice were immunized with the antigen and then three days later 1 x 10^3 tumor cells were infused. The second schedule was similar to the first, except tumor transplantation was given one day after antigen immunization. The third schedule was essentially a reversal of the first, in which the tumor cells were now injected three days before antigen immunization. All three schedules produced very similar results, and the data reported in this paper is based on the second immunization schedule. Regardless of the antigen, mice immunized with immunogenic doses of antigen (10 μg BL; 0.5 μg S-III) showed no detectable effect on the growth and development of the myeloma (Table 22). However, tolerogenic doses of the appropriate antigen (1 mg BL) was able to delay the growth and development of the ABPC48 myeloma cells. Mice receiving tolerogenic doses of the appropriate antigen had no detectable effect

TABLE 22

Effect of Immunogenic and Tolerogenic Doses of Antigen on the Kinetics of Myeloma Growth

BALB/c immunized with	Day 14 Spl-Wt[1]	Day 14 [^{3}H]Td[2]	Day 14 IgA[3]	Day 21 Spl-Wt	Day 21 [^{3}H]Td	Day 21 IgA	Day 28 Spl-Wt	Day 28 [^{3}H]Td	Day 28 IgA
Nil	0.15 ± 0.10	0.4 ± 0.2	<10	0.43 ± 0.15	0.9 ± 0.4	<10	0.56 ± 0.11	4.7 ± 1.3	ND
10 µg BL	0.17 ± 0.00	0.4 ± 0.1	ND	0.58 ± 0.01	1.6 ± 0.1	ND	0.58 ± 0.07	4.2 ± 0.4	430 ± 288
1 mg BL	0.14 ± 0.02	0.6 ± 0.3	ND	0.57 ± 0.18	1.1 ± 0.2	ND	0.33 ± 0.14	0.7 ± 0.1	<10
0.5 µg S-III	0.15 ± 0.10	0.8 ± 0.1	ND	0.26 ± 0.12	0.8 ± 0.4	ND	0.38 ± 0.12	3.9 ± 2.7	230 ± 30
1 mg S-III	0.12 ± 0.01	0.3 ± 0.1	ND	0.57 ± 0.02	0.6 ± 0.1	ND	0.53 ± 0.07	5.6 ± 0.4	232 ± 30

[1]Spleen weight (Spl-Wt) is expressed as the mean (g) ± SE.
[2]Relative proliferation of 1 x 10^5 spleen cells incubated for 4 hours at 37°C in RPMI 1640 media supplemented with glutamine and antibiotics, and the addition of 0.1 µCi [^{3}H] Thymidine ([^{3}H]Td). Results are expressed as mean (cpm x 10^{-3}) ± SE.
[3]Titer of anti-levan IgA in the sera as determined from an RIA. Results are expressed as the mean (µg/ml) ± SE.
All mice were given an i.v. injection of 1 x 10^3 ABPC48 myeloma cells.

on either the injection 1 x 10^4 or 1 x 10^5 ABPC48 myeloma cells (data not shown).

Effect of activation of normal A48Id$^+$ B cell clones on the growth of ABPC48 myeloma cells. Since it has been shown that malignant B cell clones can be influenced by the same network signals which their normal counterparts are subject to (Rohrer and Lynch, 1977; Williamson et al., 1976), it was important to study the effects of the activation of normal A48Id$^+$ B cell clones on the growth of ABPC48 myeloma cells. As previously demonstrated A48Id$^+$ anti-BL plaque forming cells could be activated by the administration at birth of anti-A48Id antibodies (0.01 - 10 μg), followed by immunization one month later with BL. The results summarized in table 23 inidicate that this treatment had no detectable effect on the growth of the ABPC48 myeloma cells.

Effect of monoclonal antibodies expressing A48- PC10 regulatory idiotopes on the growth of ABPC48 myeloma cells. Several monoclonal antibodies expressing regulatory idiotopes encoded by V_H genes derived from the V_H441-4 germ line gene family were used to induce anti-myeloma immunity. The properties of these antibodies have been described elsewhere (Victor-Kobrin et al., 1985) and the results of previous experiments are summarized in table 24.

Northern blot analysis demonstrated the ability of RNA from these monoclonal antibodies to hybridize with the V_H441-4 probe. Southern blot analysis demonstrated the Eco RI digested DNA fragments from these monoclonal antibodies contained rearranged genes derived from the V_H 441-4 family. The majority of these antibodies were able to bind two monoclonal anti-idiotype antibodies recognizing A48-UPC10 idiotypes. Six of these antibodies, like ABPC48, bind both rye levan and bacterial

TABLE 23

Effect of Normal A48Id$^+$ B Cell Clones Activated by the Administration at Birth of Minute Amounts of Anti-A48Id Antibodies on Myeloma Growth

Injected at birth with:	Immunized one month later with 10 µg BL	Injected with 10^4 ABPC48 cells	No. of mice	Day 21 Spl-Wt[1]	Day 21 [^{3}H]Td[2]	Day 21 IgA[3]
Nil	-	-	2	0.15 ± 0.03	0.4 ± 0.2	<1
Nil	-	+	4	0.80 ± 0.08	4.7 ± 0.7	15 ± 6
0.01 µg anti-A48Id	-	+	3	0.76 ± 0.02	2.4 ± 0.9	7 ± 1
0.01 µg anti-A48Id	+	+	4	0.78 ± 0.11	3.7 ± 0.6	10 ± 4
10 µg anti-A48Id	+	+	3	0.54 ± 0.06	4.2 ± 1.0	6 ± 1
0.01 µg anti-M460Id	+	+	4	0.38 ± 0.07	4.1 ± 1.7	44 ± 12

[1]Spleen weight (Spl-Wt) is expressed as the mean (g) ± SE.
[2]Relative proliferation of 1 x 10^5 spleen cells incubated for 4 hours at 37°C in RPMI 1640 media supplemented with glutamine and antibiotics, and the addition of 0.1 µCi [^{3}H] Thymidine ([^{3}H]Td). Results are expressed as mean (cpm x 10^{-3}) ± S.E.
[3]Titer of anti-levan IgA in the sera as determined from an RIA. Results are expressed as the mean (µg/ml) ± SE.

TABLE 24

Summary of Immunochemical and Molecular Properties of Monoclonal Antibodies Expressing A48-UPC10 Regulatory Idiotopes

Monoclonal antibody	A48-UPC10Id[1] recognized by IDA10	10-1	Antigen[2] binding specificity β2→6	β2→6, β2→1	V Genes[3] Vκ10	Vh441-4
ABPC48	+++	+	+++++	+++	+	+
UPC10	+	+++++	++	+++++	++++	+
MOPC173	-	±+	-	-	++++	+
MOPC460	-	-	-	-	ND	-
1-5-1	±	±	+	+++	++++	+
2-1-3	-	+	+++±	+++	ND	+
2-28-9	±	++	+	+±	ND	+
3-9-9	+±	+	-	-	-	+
3-14-9	++	+±	+++++	±	ND	+
3-27-6	+++	+±	+±	-	+±	+
3-101-14	+	±	-	-	-	+
AIDA10/16	+	+±	-	+	+	+
AIDA10/21	+±	+±	-	-	±	+
AIDA23/2	+++++	+±	-	-	-	+
AIDA23/3	+++++	+±	-	-	-	+

[1]IDA10 and 10-1 are monoclonal anti-A48Id and anti-UPC10Id antibodies respectively. Relative binding was determined from an RIA.

[2]Relative binding was recorded from an RIA in which the appropriate antigen was on the plate, then the monoclonal antibodies were added; binding was measured using ^{125}I-anti-κ antibodies. β2→6:rye levan; β2→6, β2→1: BL.

[3]^{32}P nick translated 441-4 gene probe was hybridized to nitrocellulose filters containing cytoplasmic RNA from cell lysates of the above mentioned monoclonal antibodies. Relative binding of alkaline phosphatase labeled monoclonal antibodies to plates coated with anti-Vκ10 antibodies in an ELISA. ND: not done.

levan. Rye levan is a β2→6 polyfructosan, and bacterial levan is a β2→6 polyfructosan with β2→1 branch points. Thus, these six monoclonal antibodies probably recognize β2→6 fructosan epitopes. One monoclonal was only able to bind rye levan, and one other only bacterial levan. The latter probably recognizes a β2→6, β2→1 conformational epitope.

These monoclonal antibodies were used for immunization in their native conformation. Thus, they were neither reduced and alkylated, nor coupled to carriers in order to make them more immunogenic. Therefore, immunization with these antibodies produced low titers of anti-A48Id antibodies (Table 25). Four patterns of immunity are observed based on the survival of the animals immunized (Table 26). The first group resembled the control group which is represented by normal nonimmunized mice infused with 1 x 10^4 ABPC48 myeloma cells. These include UPC10, 2-1-3, and AIDA23/2. These monoclonal antibodies showed no protective effect against myeloma growth. The second group showed a slight increase in survival and is represented by immunization with MOPC173 and 3-9-9. The third group exhibited a strong anti-tumor immunity and is represented by immunization with ABPC48, 1-5-1, 3-101-14, and AIDA10/21. However, five of the monoclonal antibodies were able to induce a long lasting anti-myeloma immunity (i.e. 2-28-9, 3-14-9, 3-27-6, AIDA10/16 and AIDA23/3). Mice in this last group survived for more than 95 days. Survival did not appear to be due to an increase in the titer of anti-A48Id antibodies that may have occurred from the exposure of suppressed, but not eliminated, ABPC48 myeloma cells (Table 25). One would not expect this to be important because Frikke et al. (1977) demonstrated that there was no correlation between the titer of anti-idiotype antibodies and the degree of

TABLE 25

Titers of Anti-A48Id and Anti-AIDA23/3 in the Sera of Immunized Mice

Monoclonal antibody	Titer of[1] anti-A48	Titer of[2] anti-AIDA23/3	Titer of[3] anti-A48
ABPC48	1.0 ± 0.3	0.5 ± 0.4	------
UPC10	0.3 ± 0.2	0.1 ± 0.1	------
MOPC173	0.5 ± 0.4	0.8 ± 0.5	------
MOPC460	0.4 ± 0.2	0	------
1-5-1	0.9 ± 0.7	0	------
2-1-3	0.2 ± 0.2	0.1 ± 0.1	------
2-28-9	0	0.4 ± 0.2	0.5 ± 0.2
3-9-9	0.3 ± 0.2	0.3 ± 0.1	------
3-14-9	0.1 ± 0.1	1.3 ± 0.8	0.6 ± 0.2
3-27-6	0	0	0.6 ± 0.2
3-101-14	0	0.7 ± 0.7	------
AIDA10/16	0	0	0
AIDA10/21	0	0	------
AIDA23/2	0	1.5 ± 0.7	------
AIDA23/3	0.1 ± 0.1	0.5 ± 0.2	0

[1]Titer of anti-A48 in the sera of mice 7 days after last immunization with the monoclonal antibody listed, determined from a standard inhibition curve with IDA10 in an RIA. Results are expressed as the mean (μg/ml) ± SE.

[2]Titer of anti-AIDA23/3 in the sera of mice 7 days after last immunization with the monoclonal antibody listed, determined from a standard inhibition curve with IDA10 in an RIA. Results are expressed as the mean (μg/ml) ± SE.

[3]Titer of anti-A48 in the sera of mice 60 days after transplantation of ABPC48 myeloma cells, determined from a standard inhibition curve with IDA10 in an ELISA. Results are expressed as the mean (μg/ml) ± SE.

TABLE 26

Effect of Monoclonal Antibodies Expressing A48-UPC10
Regulatory idiotopes on the growth of ABPC48 Myeloma Cells

Monoclonal antibody	Injected with 10^4 ABPC48 cells	Survival[1]	Spl-Wt[2]	[^{3}H]Td[3]	IgA[4]
Nil	-	------	0.10 ± 0.00	0.2 ± 0.0	0
Nil	+	21 ± 2	ND	ND	ND
ABPC48	+	41 ± 5	0.86 ± 0.04	3.0 ± 0.4	280 ± 160
UPC10	+	27 ± 4	0.54 ± 0.02	2.9 ± 0.3	480 ± 420
MOPC173	+	31 ± 3	0.64 ± 0.05	3.2 ± 0.7	1,420 ± 940
1-5-1	+	40 ± 6	0.75 ± 0.10	2.9 ± 0.8	260 ± 100
2-1-3	+	24 ± 2	0.66 ± 0.02	4.1 ± 1.2	90 ± 40
2-28-9	+	>95	ND	ND	170 ± 140
3-9-9	+	30 ± 4	0.64 ± 0.07	4.0 ± 0.5	60 ± 30
3-14-9	+	>95	ND	ND	40 ± 20
3-27-6	+	>95	ND	ND	10 ± 10
3-101-14	+	41 ± 5	0.86 ± 0.20	3.7 ± 0.9	1,990 ± 1,960
AIDA10/16	+	>95	ND	ND	60 ± 50
AIDA10/21	+	51 ± 5	0.81 ± 0.16	3.3 ± 1.0	210 ± 140
AIDA23/2	+	26 ± 2	0.52 ± 0.06	2.7 ± 0.4	0
AIDA23/3	+	>95	ND	ND	80 ± 70

[1]Survival is recorded as the mean (days) ± SE.

[2]Spleen weight (Spl-Wt) is expressed as the mean (g) ± SE.

[3]Relative proliferation of 1 x 10^5 spleen cells incubated for 4 hours at 37°C in RPMI 1640 media supplemented with glutamine and antibiotics, and the addition of 0.1 μCi [^{3}H] thymidine ([^{3}H]Td). Results are expressed as the mean (cpm x 10^{-3}) ± SE.

[4]Titer of anti-levan IgA in the sera as determined from an ELISA. Results are expressed as the mean (μg/ml) SE. All titers were recorded from the sera of mice at the time of sacrifice except for those which survived more than 95 days. For the mice surviving more than 95 days, a blood sample was taken at day 60, and the serum was used in this experiment. The value shown for mice immunized with 2-28-9 represents the results from 3 mice. The other two mice had values of 960 μg/ml. This result though may be extraneous, since the sera were highly hemolyzed, and this often leads to inaccurate measurements.

ND: not done.

protection against myeloma growth.

In order to investigate whether survival for more than 95 days was an indication of permanent immunity, the mice were reinjected with 1 x 10^5 ABPC48 myeloma cells (Table 27). However, upon second challenge with the myeloma cells, none of the mice showed any resistance to the tumor. This observation may be explained in two ways. The first and most simple is that 1 x 10^5 cells was above the threshold for which the immunity was effective. It is possible that 1 x 10^4 myeloma cells was the highest limit to which this immunization schedule could handle. Second, it is more likely that resistance to challenge was due to the action of specific T cells. There is precedent for involving T cells as the component responsible for tumor resistance (Rohrer et al., 1979; Abbas et al., 1980a; Milburn and Lynch, 1982; Abbas et al., 1980c; Snodgrass et al., 1981). However, due to the low immunogenicity of the immunizing proteins, effector T cell memory was probably not elicited, and only the immediate effector T cell function was able to combat and eliminate the initial challenge with 1 x 10^4 ABPC48 myeloma cells.

Idiotype specificity of anti-myeloma immunity. It was important to investigate if the survival for more than 95 days was idiotype specific. Idiotype specificity was determined in two ways. First, mice were immunized with MOPC460 myeloma protein. MOPC460 is an IgA which binds the haptens DNP and TNP. It expresses a different idiotype than ABPC48, and is derived from a different germ line gene family. These mice were then challenged with 1 x 10^4 ABPC48 myeloma cells. Table 28 shows that MOPC460 immunization had no effect on the growth of the ABPC48 myeloma cells. Thus, the immunity previously described could not be due to a general reaction of the immunization schedule

TABLE 27

Determination of the Permanence of Anti-Myeloma Immunity

Monoclonal antibody	Injected with[1] 10^5 ABPC48 cells	Survival[2]	Spl-Wt[3]	[^{3}H]Td[4]	IgA[5]
Nil	-	------	0.10 ± 0.00	0.2 ± 0.0	0
Nil	+	14 ± 0	0.79 ± 0.03	3.7 ± 0.5	40 ± 40
2-28-9	+	15 ± 0	1.17 ± 0.14	3.1 ± 0.2	20 ± 20
3-14-9	+	17 ± 1	1.13 ± 0.12	3.2 ± 0.3	390 ± 270
3-27-6	+	15 ± 0	1.31 ± 0.04	4.7 ± 1.3	18,890 ± 16,930
AIDA10/16	+	16 ± 1	1.08 ± 0.22	4.8 ± 0.9	2,890 ± 1,520
AIDA23/3	+	16 ± 0	1.39 ± 0.07	3.7 ± 0.3	1,820 ± 1,060

[1]All the mice treated with the monoclonal antibodies in this experiment survived the initial challenge with 10^4 ABPC48 myeloma cells before receiving the injection with 10^5 ABPC48 myeloma cells.

[2]Survival is recorded as the mean (days) ± SE after the injection of 10^5 ABPC48 myeloma cells.

[3]Spleen weight (Spl-Wt) is expressed as the mean (g) ± SE.

[4]Relative proliferation of 1 x 10^5 spleen cells incubated for 4 hours at 37°C in RPMI 1640 media supplemented with glutamine and antibiotics, and the addition of 0.1 µCi [^{3}H] thymidine ([^{3}H]Td). Results are expressed as the mean x 10^{-3}) + SE.

[5]Titer of anti-levan IgA in the sera determined from an ELISA. Results are expressed as the mean (µg/ml) ± SE.

TABLE 28

Idiotype Specificity of Anti-Myeloma Immunity

Monoclonal antibody	Myeloma cells injected	Survival[1]	Spl-Wt[2]	[^{3}H]Td[3]	IgA[4]
Nil	10^4 ABPC48	21 ± 2	ND	ND	
MOPC460	10^4 ABPC48	25 ± 3	0.62 ± 0.08	3.1 ± 0.6	360 ± 340
		Number of PCFU-s[5]			
Nil	4×10^4 MOPC315	11.5 ± 0.5			
3-27-6	4×10^4 MOPC315	10.8 ± 0.6			
AIDA10/16	4×10^4 MOPC315	4.0 ± 1.2			
AIDA23/3	4×10^4 MOPC315	9.5 ± 1.2			

[1]Survival is recorded as the mean (days) ± SE.
[2]Spleen weight (Spl-Wt) is expressed as the mean (g) ± SE.
[3]Relative proliferation of 1×10^5 spleen cells incubated for 4 hours at 37°C in RPMI 1640 media supplemented with glutamine and antibiotics, and the addition of 0.1 µCi [^{3}H] thymidine ([^{3}H]Td). Results are expressed as the mean (cpm $\times 10^{-3}$) + SE.
[4]Titer of anti-levan IgA in the sera as determined from an ELISA. Results are expressed as the mean (µg/ml) ± SE.
[5]Fourteen days after injection mice were sacrificed and their spleens were removed and placed in vials containing Bouin's fixative. After 24 h the spleens were examined and all visible and distinct nodules were counted. Each nodule represents one plasmacytoma colony forming unit (PCFU). The results are expressed as the mean number of PCFU-s ± SE.
ND: not done

which utilized FCA. Second, three of the five monoclonal antibodies which provided a long lasting immunity were used to immunize another set of animals. These mice were then challenged with 4×10^4 MOPC315 spleen adapted myeloma cells. The results (Table 28) demonstrate that these antibodies could not render mice resistant to the MOPC315 myeloma, since similar numbers of PFCU-s were obtained with nonimmunized mice.

DISCUSSION

The balance between clones as well as communications which are established between various subsets of regulatory T cells based on fragile idiotypic links, can easily be perturbed by antigens, idiotypes, and anti-idiotype antibodies. This paper presents results that show that the administration of A48Id monoclonal protien at birth has a profound effect on the expression of the A48Id$^+$ anti-β2→6 fructosan precursor antibody-forming cells. Neonatal treatment as well as the treatment of mice during the first weeks after birth with monoclonal protein led to the A48Id dominance of the anti-β2→6 fructosan antibody response. Once the dominant β2→1 fructosan-reactive clones emerge 28 days after birth, one can no longer activate A48Id clones. Indeed, no A48Id-bearing antibodies were detected in 4-week-old BALB/c mice treated with A48 monoclonal protein and immunized 1 month later with BL.

Two groups of findings demonstrate that this activation is idiotype as well as antigen dependent and specific: (a) the administration at birth of M460 monoclonal protein led to an increase of M460Id component of anti-TNP antibodies, but not of the A48Id component of anti-β2→6 fructosan response; (b) only the administration with BL of 1-month-old BALB/c mice treated at birth with A48 protein elicited an A48Id$^+$ BL-specific response, whereas immunization with TNP-Ficoll does not. These results are in agreement with those reported by Reth et al. (1981), which showed that the increase of (4-hydroxy-3-nitrophenyl)acetyl(NP) V_HbId component after injection of NP-binding monoclonal antibodies in adult mice requires specific antigenic stimulation. A small but significant stimulation of the

β2→1 fructosan-reactive clones was observed in BALB/c mice that were injected at birth with A48 monoclonal protein and challenged 1 month later with In-BA. This observation is quite surprising as we have previously shown a significant ontogenic delay of the expression of these clones in that they only become dominant in 6-week-old mice (Bona et al., 1978). However, the premature activation of these clones can be exclusively related to a more profound alteration of the steady-state balance at birth maintained between the clones by the administration of A48 monoclonal protein. Bona et al. (1979c) previously described an "indirect idiotype phenomenon" based on the observation, made in nude mice, that treatment with anti-E109IdX antibodies resulted in a long-lasting suppression of the anti-β2→6 fructosan response in addition to the appearance of $A48Id^+$ antibodies. Preliminary data regarding the analysis of antigen-binding specificity of several monoclonal antibodies obtained from mice treated at birth with A48 monoclonal protein show that they exhibit a high binding activity to β2→6 fructosan epitopes, but they also bound β2→1 fructosan epitopes. These results would suggest that the products of clones that were activated by manipulation of the immune network differ from those activated in adult mice after immunization with BL as well as from In-binding myeloma proteins that exhibit a higher binding activity for β2→1 fructosan epitopes than for β2→6 fructosan epitopes (Lieberman et al., 1975).

The A48 idiotype expressed on A48 myeloma protein is composed of several idiotopes (Legrain et al., 1981); some of them are shared by U10 monoclonal protein and β2→6 fructosan-binding monoclonal antibodies that we have recently obtained. The treatment at birth with U10

and 3-76-42, but not with 3-76-36, monoclonal antibodies led to the activation of A48Id. These results support the previous concept concerning the idiotype network in that only few idiotopes, that is, regulatory idiotopes, function in an autologous system. It was proposed that regulatory idiotypes might be a feature only of those idiotypes that are capable of becoming dominant idiotypes, possibly because it is these determinants that call for the T cell regulatory responses (Paul and Bona, 1982; Bona et al., 1981).

The results show that the activation of A48Id silent clones depends on the expansion of A48Id-specific helper T cells. The infusion of B cells from A48-treated mice into irradiated mice does not cause the production of A48Id-bearing antibodies in response to immunization with BL. This clearly demonstrated that A48Id-bearing immunoglobulins have no effect on B cells that carry the A48 immunoblobulin receptor. By contrast, the infusion of irradiated animals with a mixture of T and B cells from A48-treated mice induced the occurrence of A48Id-bearing antibodies. These T cells express Lyt-1.2 alloantigen and their activity cannot be overcome by putative naturally occurring A48Id-specific suppressor T cells (Lieberman et al., 1979), at least at a 1:1 cell ratio. Because we failed to enrich for the putative A48Id-specific helper T cells on A48-coated dishes, we studied the specifity of the receptor of these cells in nude mice. In these experiments we found that nude mice infused with T cells from animals treated at birth with A48 contained a discrete subset of cells that recognize the A48 idiotypes. When these cells were infused into nude BALB/c mice, they rendered them capable of mounting an anti-TNP response upon immunization with A48-TNP conjugate. This subset of

cells that exert their helper effect through an antigen bridge were also sensitive to A48 and anti-Lyt-1.2 antiserum plus complement treatment. It is conceivable that the antigen bridge through which these T cells exert their helper effects in A48-treated animals can be composed of bacterial levan-A48Id$^+$ antibody complex. A precedent for T cells that exert their effect through an antigen bridge in which the antigen is bound to an immunoglobulin receptor which is simultaneously recognized by the T cell receptor were previously described in the MOPC315 system. In this system, it was shown that BALB/c splenocytes specific for TNP-modified syngeneic cells could induce the lysis of MOPC315 cells in the presence of soluble TNP conjugates (Abbas et al., 1980c). The possibility remains, however, that they may be distinct from the subset of cells observed to help in the expression of A48Id$^+$ BL-specific response in lethally irradiated mice. Therefore, our present results suggest that the treatment with A48 monoclonal protein at birth activates A48Id-specific helper T cells that select for the expansion, subsequent to antigenic stimulation, those clones that express A48Id on their immunoglobulin receptors. Attempts to clone these helper T cells will provide information on their fine idiotype specificty, target, and phenotype, as well as major histocompatibility complex restriction.

One may ask whether clonal activation by parenteral administration of idiotype at birth has any physiological significance. It is well known that significant amounts of IdX-bearing antibodies specific for environmental antigens can be detected in the sera of nonimmunized adult mice (Potter, 1977). Furthermore, the precursors responsible for mounting these antibody responses can be detected in newborn mice

by precursor frequency analysis (Klinman et al., 1976). In addition, various investigators have observed that vigorous immune responses for environmental antigens (e.g., phosphorylcholine, β2→6 fructosan, α,1-3 dextran, galactan, arsonate, TNP coupled to TI-1 antigens) can be elicited in 1-7-day-old mice (Bona, 1981). Therefore, the passive influx of maternal idiotypes in the immune system of the embryo or newborn, by placenta or colostrum transfer, can influence the idiotype distribution by favoring the expression of those clones that were dominant in the maternal immune system. In the rabbit, Wikler et al. (1980) have also shown that progeny can learn to make antibodies bearing the idiotype of another rabbit by maternal transfer of anti-(anti-idiotype) antibodies. In physiological terms it is extremely advantageous to have the immune experience of the mother transmitted to the next generation with the resulting expansion of precursors that are specific for a response against commonly encountered pathogens prevalent in the species. This is particularly important in a given period of the postnatal life when the concentration of antibodies provided from the mother starts to decline. Absence of an idiotype-induced expansion can clearly explain the observation that exposure of the human population to new pathogens that had not been encountered in previous generations, frequently results in widespread epidemics (e.g., influenza pandemics after a major antigenic shift of the influenza virus hemagglutinins) (Kilbourne, 1978).

In theoretical terms, this concept would redefine the notion of inheritance of immunity as not only a passive acquisition of maternal antibodies, but also a priming of the immune system for specific

immune responses. This would suggest that once an immune response is initiated by antigenic stimulation, the actual antigen-response clones that will proliferate can be determined by the idiotypic repertoire of the mother that has been established by an earlier encounter with the same antigen.

The study of the genetic control of the expression of A48Id has clearly shown that the expression of A48Id is not dependent upon MHC or IghC gene complexes. Indeed A48Id$^+$ clones were activated in BALB/c as well as in BALB.B and BALB.K mice. These two strains of mice are BALB/c congenic strains that differ only in the MHC gene complex. The results obtained in BAB.14 and CAL.20 indicated that the activation of A48Id$^+$ clones is not associated with the IghC^a haplotype. Furthermore, the presence of A48Id$^+$ anti-β2→6 polyfructosan antibodies in CAL.20 and CCB.R4 mice clearly indicates that the A48 idiotype is not solely a marker of IghV^a genes. The presence of A48Id in CAL.20 mice is in agreement with the data that showed that the IdX B,A, and G of β2→1 polyfructosan binding myeloma proteins, originating in BALB/c, were expressed on antibodies induced by immunization with BL in CAL.20 mice (Lieberman et al., 1979).

One may ask about the physiological significance of the activation of A48Id silent clones through the manipulation of a steady state network by the administration at birth of idiotype or anti-idiotypic antibodies. One might deduce from our previous studies that the administration of A48Id during the neonatal period is critical for the subsequent dominance of the anti-levan response by A48Id$^+$ clones. Based on these results, we propose the hypothesis that the passive influx of maternal idiotypes to the embryo or newborn across the

placenta or through the colostrum, can influence the idiotype distribution in the newborn by favoring the expression of these clones that were dominant in the maternal immune system. The results obtained in the UPC10 system lend strong support to this hypothesis. The parenteral administration of UPC10 monoclonal protein into mice before and during pregnancy led to the activation of U10Id$^+$ clones that otherwise were not expressed during the conventional anti-levan immune response.

The expression of the U10Id$^+$ clones is also independent of MHC and IghC gene complexes. By contrast, however, to the activation of A48Id clones subsequent to parenteral administration of A48Id or anti-A48Id antibodies at birth, the activation of U10Id clones by in utero exposure to UPC10 monoclonal protein, is dependent on IghV^a genes. Indeed, the CCB.R4 progeny obtained from females injected with UPC10 did not express the UPC10 idiotype. The lack of UPC10Id$^+$ anti-β2→6 polyfructosan antibodies in CCB.R4 mice can be related to the induction of suppressor cells specific for 173-UPC10 V_H antigenic determinants borne by several IghV^a proteins belonging to various V_H subgroups that can prevent the activation of UPC10 (Bosma et al., 1977). These determinants can be recognized as foreign antigenic determinants of CCB.R4 mice bearing IghV^b genes. Alternatively, the expression of U10Id can require a light chain of a haplotype that is not provided by the CCB.R4 mice.

Immunization of BALB/c mice with BL a β2→6 polyfructosan with β2→1 branch points, leads to a vigorous T-independent antibody response. Two families of clones are involved in this response. The first family of clones is specific for the β2→6 fructosan linkage and

does not express the idiotype of A48 and U10 myeloma proteins that are specific for the β2→6 linkage. An A48Id$^+$ response was detected only in CXBJ recombinant inbred strains of mice, among the various strains that were investigated. The synthesis of anti-β2→6 fructosan antibodies is independent of IghC genes and occurs early in ontogeny. The second family of clones produces antibodies specific for β2→1 fructosan linkages. These antibodies share the IdX G, B, and A of In-binding myeloma proteins. The expression of IdX is associated with the IghC^a allotype. The clones producing these antibodies belong to the Lyb-5$^+$ subset and appear late in development.

In this presentation we present further results of the effect of the adminstration at birth of minute amounts of anti-A48Id antibodies on the activation of normally silent A48Id$^+$ anti-BL B cells. The priming at birth with minute amounts of anti-idiotypic antibodies has a long-lasting effect, since an A48Id$^+$ response was observed even in 12-week-old mice. The long-lasting effect is not related to the persistence of anti-idiotypic antibodies. Radiolabelled monoclonal proteins injected at birth are completely cleared from the blood by 30 days. The transfer of an A48Id$^+$ response with highly purified B cells from mice treated at birth with anti-A48Id antibodies and then infused into lethally irradiated mice, suggests that the activation of precursors of A48Id$^+$ secreting cells is T-independent under these conditions. It might be envisioned that the interaction of anti-A48Id antibodies with cellular receptors bearing the A48Id mimics the binding of a T-independent antigen to a B cell receptor, and that in the absence of T cell help leads to the activation of that B cell. In a general sense, globular proteins such as a mouse immunoglobulin

bearing a combining site with anti-idiotypic specificity is very likely to behave as a "thymus independent" antigen.

Although the anti-A48Id antibodies can prime, independently of T cell help, the A48Id-bearing precursors, their effect is not related to a mitogen-like effect. No anti-β2→6 fructosan response was observed in BALB/c mice treated at birth with anti-A48Id antibodies that were not challenged with BL or that were challenged with TNP-Ficoll. In several experimental systems it was clearly shown that the enhancement of the idiotype response after the administration of idiotype (Kelsoe et al., 1981; Heyman et al., 1982) or anti-idiotype antibodies (Bona et al., 1979a; Bona et al., 1981; Kelsoe et al., 1981) requires antigenic challenge.

The most striking observation in our study was the ability of a monoclonal anti-A48Id antibody to elicit an anti-BL response in mice treated at birth with either polyclonal anti-A48Id antibodies or BL. Such mice do not develop an anti-BL response without antigen or anti-idiotype challenge. The results of PFC and IEF data show that whereas BL-immunized 1-month-old mice develop only an anti-β2→6 fructosan response lacking A48 and U10 idiotypes and exhibiting typical BALB/c spectrotypes, the 3-month-old mice developed a vigorous anti-β2→1 and β2→6 fructosan response, expressed the W3082IdX, and exhibited a typical BALB/c anti-BL spectrotype and anti-In J606-like spectrotype. The administration at birth of minute amounts of anti-A48Id antibodies profoundly altered the clonal expression. Thus, the challenge with BL of 1-month-old mice led to the activation of A48 and U10Id$^+$ anti-BL clones which correspond to a U10-like spectrotype. Furthermore, immunization with BL of 3-month-old mice still led to the

activation of A48 and U10Id anti-BL clones, but the product of these clones corresponds to a spectrotype that was observed in CXBJ mice, the single strain that developed an A48-Id$^+$ response. CXBJ spectrotype is characterized by five bands focusing in the same region as J606 but binds BL rather than In.

The challenge with 17-38 monoclonal anti-A48Id antibody of mice treated at birth with anti-A48Id antibodies or BL presented a completely different picture. In 1-month-old mice the anti-BL antibodies failed to express the A48 and U10Id idiotypes and the spectrotype is of the type found in adult BALB/c mice. In 3-month-old mice, in addition to an A48Id negative and U10Id negative anti-BL response, a vigorous In W3082Id$^+$ PFC response was observed. These responses correspond to a BALB/c BL spectrotype and In J606 spectrotypes as seen in normal 3-month-old mice. These findings indicate that; (a) the challenge with antigen can be replaced by one out of six monoclonal anti-A48Id antibodies, clearly demonstrating that not all anti-A48Id antibodies exhibit antigen-like properties. The ability of 17-38 monoclonal antibody to stimulate anti-$\beta 2 \rightarrow 6$ and $\beta 2 \rightarrow 1$ clones cannot be related to a particular isotype or its avidity since other IgM monoclonal anti-A48Id antibodies lack this property. The pentameric structure of IgM confers a high avidity to the antibodies belonging to this class of immunoglobulin; and (b) there is a fundamental difference between the challenge with antigen and the challenge with 17-38 monoclonal antibody. Whereas the challenge with antigen led to the activation of the A48 and U10Id$^+$ component of the anti-BL response ($\approx$50% in 1-and 3-month old mice), the challenge with 17-38 monoclonal antibody led to the suppression of A48 and U10Id$^+$

clones and to the activation of A48Id negative anti-β2→6 fructosan-reactive clones in 1-month-old mice. In 3-month-old mice there was an activation of W3082IdX$^+$ anti-β2→6 and β2→1 fructosan-reactive clones. One possible explanation of the "antigen like" property of this monoclonal antibody, is that it actually represents a homobody (Lindenmann, 1979), i.e., an anti-idiotype antibody carrying the internal image of the antigen.

Anti-idiotype idiotype antibodies carrying the internal images of the antigens (i.e., homobodies) were reported in several systems. The majority of the studies, carried out on homobodies, were focused on their ability to inhibit the binding of antigen to its corresponding receptor (e.g., insulin, Sege and Peterson, 1978a; retinol, Sege and Peterson, 1978b, catecholamine, Schreiber et al., 1980; formylpeptide, Marasco and Becker, 1982; reovirus hemagglutinin, Nepom et al., 1982; murine anti-idiotypic antibody displaying anti-human γ-globulin activity, Bona et al., 1982) or to mimic the antigen effects after the binding of homobodies to the "antigen cell receptor" (e.g., insulin, Sege and Peterson, 1978a; alprenolol, Schreiber et al., 1980). However, it is obvious that homobodies like all anti-idiotype antibodies (Ab2 = p2i2) exhibit two functions, because through their paratopes (p2), they can bind to clones bearing the corresponding idiotype and through their idiotype (i2), they can stimulate other clones. We were fortunate that in our system we were able to analyze these two functions of 17-38 anti-A48Id monoclonal antibody, since the administration at birth of minute amounts of anti-A48Id antibodies induces a long-lasting activation of A48Id$^+$ anti-β2→6 fructosan-reactive clones and the natural ontogenic delay of the expression of

W3082IdX anti-β2→6 and β2→1 fructosan-reactive clones.

Results suggest that through its idiotype(s) (i2), the 17-38 monoclonal antibody activated the proliferation of A48Id negative or W3082IdX$^+$ clones (Abl = $pl^a il^a$[Ab, antibody; p, paratope; i, idiotope]) and through its paratope occluded the expression of A48Id$^+$ clones (Abl = $pl^b il^a$) (Fig. 10). Recently Jerne et al. (1982) classified anti-idiotype antibodies into two categories: Ab2α, the anti-idiotype antibodies directed against the conventional idiotopes of Abl, and Ab2β, the anti-idiotype antibodies carrying the internal image of the antigen. Our results show that a monoclonal anti-idiotype antibody carrying the internal image of the antigen is bifunctional because, by its paratope, it interacts with the immunoglobulin receptor of clones bearing the corresponding idiotype and by its idiotope can interact with the immunoglobulin receptor of clones expressing unrelated idiotypes. No available data exists on the structural correlates of the homobodies. It is very likely that the internal images are only putative copies (i.e., topochemical copies) of the antigen, a product of steric resemblance and not of identity in amino acid sequence. Crystallographic studies of Fab fragments of several anti-A48Id monoclonal antibodies (including 17-38) will shed light on the shapes of variable regions of antibodies that mimic the antigen.

It has been demonstrated that the antigen could inhibit the growth of myeloma cells in vitro. Our in vivo studies clearly demonstrated that immunogenic doses of bacterial levan does not effect the growth of the myeloma, whereas a tolerogenic dose, substantially delayed the development of the myeloma. It is generally accepted

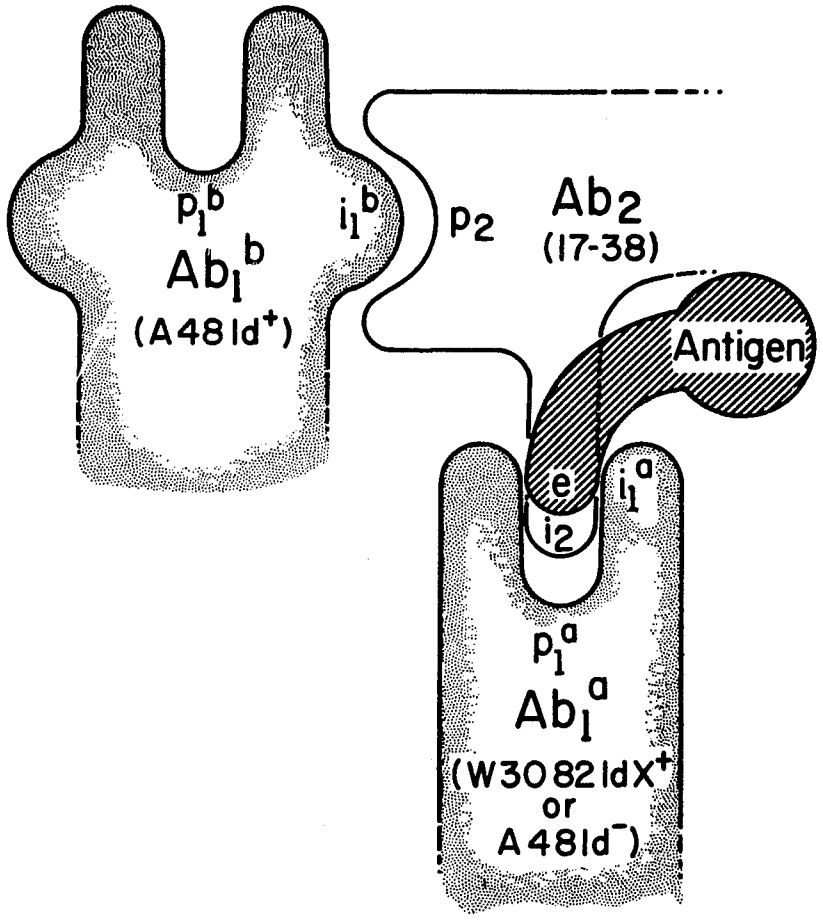

FIGURE 10. Functional dualism of a homobody. Ab, antibody; p, paratope; i, idiotope; e, epitope.

that B cells alone are involved in high dose tolerance induced by polysaccharide antigens such as levan (Kotlarski et al., 1973).

Therefore, it appears that the binding of antigen, which is available in high amounts to the immunoglobulin receptor of myeloma cells, can deliver a negative signal. This negative signal not only affects immunoglobulin secretion as in normal cells, but also affects the growth of the myeloma cells. However, this "tolerant state" is of short duration, since the clearance of antigen by antibodies produced by normal $\beta 2 \rightarrow 6$ fructosan reactive clones, or by the appearance of new tumor cells phenotypically different from the injected pool of cells, is responsible for tumor development despite a significant delay.

The expansion of normal B cell clones bearing A48 idiotopes of ABPC48 myeloma cells through the administration at birth of minute amounts of anti-A48Id antibodies, has no detectable effect on the growth of ABPC48 myeloma cells. These results were somewhat expected, since previous experiments have shown that the expansion of $A48Id^+$ B cells is related to a direct effect of minute amounts of anti-idiotype antibodies on B cells. There have been several reports demonstrating that in vitro incubation of murine myeloma cells, or in vivo administration of anti-idiotype antibodies before transfer of the myeloma, can influence the growth of the tumor (Bridges et al., 1984). Similarly, regression or long term remission of tumor growth has been observed with human B cell malignancies after the administration of anti-idiotype antibodies in high amounts (Miller et al., 1982; Meeker et al., 1985b). Since the activation of B cell clones sharing the idiotypes of the myelomas show no signs of influencing tumor growth, other mechanisms must be involved.

Studies investigating the effect of anti-idiotype therapy, in both murine and human systems, indicate that the selection for idiotype negative variants occurs after the administration of large doses of anti-idiotype antibodies. These variants are the cells responsible for escaping the effects of anti-idiotype therapy. In the murine system, Bridges et al. (1984) observed the appearance of MOPC460 variants lacking the immunoglobulin receptor on the surface of these cells, after the administration of monoclonal anti-460Id antibodies. Likewise, Meeker et al. (1985a) described two patients with malignant B cell lymphomas in which escape from anti-idiotype therapy resulted from the selection of variants using the same V_H and V_L genes, and exhibiting the same glycosylation pattern as the parent tumor, but lacking the idiotypic determinants. The selection of these variants can be easily explained, since it was shown that in the absence of antigenic pressure, 10^{-2} to 10^{-8} variants arise in cultured myeloma cell lines (Yelton and Scharff, 1982). The mutation rate which can affect the expression of idiotype on B cells expanded in vivo after immunization is about 10^{-3} per base pair per cell division (McKean et al., 1984).

Our most important observation was the idiotypic immunity elicited by immunization with monoclonal antibodies sharing ABPC48 regulatory idiotopes. Our previous investigations of the A48 idiotypic pathway led us to delineate a special category of idiotopes that we designated regulatory idiotopes. Regulatory idiotopes differ from conventional idiotopes by several properties:

a) they are autoimmunogenic, enabling them to break self tolerance and to stimulate anti-idiotype cells after self recognition;

b) they are markers of germ line V genes;

c) they can be shared by antibodies with various specificities;

d) they are able to stimulate the expansion of regulatory T cells. Thus, it was important to assess the effect these monoclonal antibodies could have on the growth of ABPC48 myeloma cells in vivo. For this study we selected from our panel of A48Id$^+$ monoclonal antibodies, a group bearing V_H genes derived from the V_H441-4 germ line gene family, some bearing the Vκ1O light chain of UPC1O, and the ability to react with two monoclonal anti-A48-UPC1O idiotype antibodies. Among 14 antibodies, 5 had affinities for both rye and bacterial levan (like ABPC48 and UPC1O); one had an affinity only for bacterial levan, probably recognizing a $\beta 2\rightarrow 6$, $\beta 2\rightarrow 1$ conformational determinant on BL; one displayed an affinity only for rye levan; 7 lacked any fructosan binding activity. Interestingly, among the last group, 5 were able to bind $\beta 1\rightarrow 6$ galactan (Victor-Kobrin et al., 1985). The binding to galactan was explained via the regulatory idiotype concept, in which regulation of clones derived from another member of the V_H441-4 family (i.e. V_HX24), the family of V_H genes encoding for galactan antibodies (Brodeur and Riblet, 1984), could share regulatory idiotopes and have different antigen specificities.

Immunization of BALB/c mice with these unmodified monoclonal antibodies elicited the synthesis of a small amount of anti-idiotype antibodies capable of inhibiting the binding of IDA1O (anti-A48Id) to A48, or IDA1O to AIDA23/3 (anti-anti-A48Id) monoclonal antibodies. This clearly indicates that these monoclonal antibodies are autoimmunogenic. However, important differences were observed with respect to their effect on the growth of ABPC48 myeloma cells. Three

monoclonal antibodies (i.e. UPC10, 2-1-3 and AIDA23/3) were not capable of eliciting immunity, since the mean of survival after the tumor graft was similar to the control groups (i.e. nonimmunized or immunized with MOPC460, which expresses a different idiotype and is derived from a different V_H germ line gene). Two of the monoclonal antibodies (i.e. MOPC173 and 3-9-9) caused a small delay in the growth of the tumor, and four monoclonal antibodies (i.e. ABPC48, 1-5-1, 3-101-14 and AIDA10/21) provided a more distinctive delay in tumor growth. Five of the monoclonal antibodies induced a very strong protection, since all the animals survived for more than 95 days after the infusion of 1×10^4 tumor cells. However, this immunity was not definitive, since the injection of 1×10^5 tumor cells at 95 days caused the animals to die. It is possible that alternate immunization schedules could improve on the longevity of this resistance. Many new approaches can be investigated, among them the effect of a booster immunization at 95 days. Also the effect lower numbers of myeloma cells have when injected 95 days.

A critical observation to arise from this study is that not all antibodies bearing immunochemically and molecularly defined regulatory idiotopes are equally efficient in providing immunity against the myeloma cells. This suggests that subtle differences exist in their structure, and that these differences play a crucial role in eliciting immunity. The V regions of these antibodies are presently being sequenced and will probably allow us to delineate a segment(s) responsible for the anti-tumor protection. Identification of such a structure will permit the synthesis of a peptide, which could then be tested for its effectiveness in eliciting anti-tumor immunity. This

could represent a new approach in idiotype therapy of human lymphocyte malignancies, preventing the occurrence of variants selected by anti-idiotype antibodies.

BIBLIOGRAPHY

Abbas, A. K., Burakoff, S. J., Gefter, M. L., and Greene, M. I. 1980a. T lymphocyte-mediated suppression of myeloma function in vitro. III. Regulation of antibody production in hybrid myeloma cells by T lymphocytes. J. Exp. Med. **152**: 969-978.

Abbas, A. K., and Klaus, G. G. B. 1977. Inhibition of antibody production in plasmacytoma cells by antigen. Eur. J. Immunol. **7**: 667-674.

Abbas, A. K., Perry, L., Bach, B. A., and Greene, M. I. 1980b. Idiotype-specific T cell immunity. I. Generation of effector and suppressor T lymphocytes reactive with myeloma idiotypic determinants. J. Immunol. **124**: 1160-1166.

Abbas, A. K., Ratnofsky, S. E., and Burakoff, S. J. 1980c. T lymphocyte mediated suppression of myeloma function in vitro. II. Evidence for regulation of hapten-binding myelomas by syngeneic hapten-specific cytolytic T lymphocytes. J. Exp. Med. **152**: 306-323.

Adinolfi, M., Glynn, A. A., Lindsay, M., and Milne, C. M. 1966. Seriological properties of γA to Escherichia coli present in human colostrum. Immunol. **10**: 517-526.

Auffray, C., Sikorav, J. L., Ollo, R., and Rougeon, F. 1981. Correlation between D region structure and antigen-binding specificity: evidence from the comparison of closely related immunoglobulin V_H sequence. Ann. Immunol. (Inst. Pasteur) **132D**: 77-88.

Bhoopalam, N., Heller, P., Meyerstein, N., and Hall, L. 1980. Effect of dextran-s (α,1-3 dextran) on the growth of plasmacytomas MOPC-104E and J558. J. Immunol. **125**: 1454-1458.

Bikoff, E. 1982. T-cell proliferative response to hapten-modified self-immunoglobulins: recognition of conjugate-specific determinants. Proc. Natl. Acad. Sci. **79**: 4156-4160.

Blomberg, B., Geckeler, W. R., and Weigert, M. 1972. Genetics of antibody response to dextran in mice. Science **177**: 178-180.

Bona, C. A. 1981. Sequential activation of V genes. In: Idiotypes and Lymphocytes, Academic Press, New York, pp. 45-58.

Bona, C. A. 1984. Idiotype and anti-idiotype regulation of antipolyfructosan response, role of regulatory idiotopes. In: The Biology of Idiotypes, Mark I. Greene and Alfred Nisonoff (eds.), Plenum Publishing Corp., New York, pp. 327-339.

Bona, C. A., Finley, S., Waters, S., and Kunkel, H. G. 1982. Anti-immunoglobulin antibodies. III. Properties of sequential anti-idiotypic antibodies to heterologous anti-γ globulins. Detection of reactivity of anti-idiotype antibodies with epitopes of Fc

fragments (homobodies) and with epitopes and idiotopes (epibodies). J. Exp. Med. **156**: 986-999.

Bona, C. A., Heber-Katz, E., and Paul, W. E. 1981. Idiotype anti-idiotype regulation. I. Immunization with a levan-binding myeloma protein leads to the appearance of auto-anti-(anti-idiotype) antibodies and to the activation of silent clones. J. Exp. Med. **153**: 951-967.

Bona, C., Hooghe, R., Cazenave, P. A., LeGuern, C., and Paul W. E. 1979a. Cellular basis of regulation of expression of idiotype II. Immunity to anti-MOPC-460 idiotype antibodies increases the level of anti-trinitrophenyl antibodies bearing 460 idiotypes. J. Exp. Med. **149**: 815-823.

Bona, C., Lieberman, R., Chien, C. C., Mond, J., House, S., Green, I., and Paul, W. E. 1978. Immune response to levan. I. Kinetics and ontogeny of anti-levan and anti-inulin antibody response and of expression of cross-reactive idiotype. J. Immunol. **120**: 1436-1442.

Bona, C., Mond, J. J., Stein, K. E., House, S., Lieberman, R., and Paul, W. E. 1979b. Immune response to levan. III. The capacity to produce anti-inulin antibodies and cross-reactive idiotypes appears late in ontogeny. J. Immunol. **123**: 1484-1490.

Bona, C., and Paul, W. E. 1979. Cellular basis of regulation of expression of idiotype. I. T-suppressor cells specific for MOPC460 idiotype regulate the expression of cells secreting anti-TNP antibodies bearing 460 idiotype. J. Exp. Med. **149**: 592-600.

Bona, C., Stein, K. E., Lieberman, R., and Paul, W. E. 1979c. Direct and indirect suppression induced by anti-idiotype antibody in the inulin-bacterial levan antigenic system. Mol. Immunol. **16**: 1093-1101.

Bosma, M. J., DeWitt, C., Hausman, S. J., Marks, R., and Potter, M. 1977. Serological distinction of heavy chain variable regions (V_H subgroups) of mouse immunoglobulins. I. Common V_H determinants on the heavy chains of mouse myeloma proteins having different binding sites. J. Exp. Med. **146**: 1041-1053.

Bridges, S. H., LeGuern, C., and Gurgo, C. 1984. Inhibition of in vivo growth of murine plasmacytoma MOPC-460 by monoclonal anti-idiotypic antibodies directed at distinct idiotypes of the immunoglobulin on the surface of MOPC-460. Cancer Res. **44**: 5051-5055.

Brient, B.J., and Nisonoff, A. 1970. Quantitative investigations of idiotypic antibodies. IV. Inhibition of specific haptens of the reaction of anti-hapten antibody with anti-idiotypic antibody. J. Exp. Med. **132**: 951-958.

Briles, D. E., and Davie, J. M. 1975. Detection of isoelectro focused antibody by autoradiography and hemolysis of antigen-coated erythrocytes. J. Immunol. Methods. **8**: 363-372.

Brodeur, P. H., and Riblet, R. 1984. The immunoglobulin heavy chain variable region (Igh-V) locus in the mouse. I. One hundred Igh-V genes comprise seven families of homologous genes. Eur. J. Immunol. **14**: 922-930.

Burnet, F. M. 1959. The Clonal Selection Theory of Acquired Immunity. Vanderbilt University Press, Nashville, pp. 49-68.

Capra, J. D., and Kehoe, J. M. 1974a. Variable region sequences of five human immunoglobulin heavy chains of the V_HIII subgroup: definitive identification of four heavy chain hypervariable regions. Proc. Natl. Acad. Sci. **71**: 845-848.

Capra, J. D., and Kehoe, J. M. 1974b. Structure of antibodies with shared idiotypy: the complete sequence of the heavy chain variable regions of two immunoglobulin M anti-gamma globulins. Proc. Natl. Acad. Sci. **71**: 4032-4036.

Capra, J. D., Klapper, D. G., Tung, A. S., and Nisonoff, A. 1976. Identical hypervariable regions in light chains of differing V subgroups. Cold Spring Harbor Symposia on Quantitative Biology **42**: 847-853.

Chien, C. C., Lieberman, R., and Inman, J. K. 1979. Preparation of functionalized derivatives of inulin: conjugation of erythrocytes for hemagglutination and plaque-forming cell assays. J. Immunol. Methods. **26**: 39-46.

Cunningham, A. J., and Szenberg, A. 1968. Further improvements in the plaque technique for detecting single antibody-forming cells. Immunol. **14**: 599-600.

Daley, M. J., Bridges, S. H., and Lynch, R. G. 1978a. Plasmacytoma spleen colonization: a sensitive, quantitative in vivo assay for idiotype-specific immune suppression of MOPC-315. J. Immunol. Methods **24**: 47-56.

Daley, M. J., Gebel, H. M., and Lynch, R. G. 1978b. Idiotype-specific transplantation resistance to MOPC-315: abrogation by post-immunization thymectomy. J. Immunol. **120**: 1620-1624.

Eisen, H. N., Sakato, N., and Hall, S. J. 1975. Myeloma proteins as tumor-specific antigens. Transplant. Proc. **7**: 209-214.

Freedman, P. M., Autry, J. R., Tokuda, S., and Williams, R. C. 1976. Tumor immunity induced by preimmunization with BALB/c mouse myeloma protein. J. Natl. Cancer Inst. **56**: 735-740.

Frikke, M. J., Bridges, S. H., and Lynch, R. G. 1977. Myeloma-specific

antibodies: studies of their properties and relationship to tumor immunity. J. Immunol. **118**: 2206-2212.

Gell, P. G. H. 1964. Anti-antibody or clone-product? Nature **201**:687-689.

Gershon, R. G. 1975. A disquisition on suppressor T cells. Transplant. Rev. **26**: 170-185.

Gleason, K., and Kohler, H. 1982. Regulatory idiotypes. T helper cells recognize a shared V_H idiotope on phosphorylcholine-specific antibodies. J. Exp. Med. **156**: 539-549.

Gold, E. R., and Fudenberg, H. H. 1967. Chromic chloride: A coupling reagent for passive hemagglutination reactions. J. Immunol. **99**: 859-866.

Goldberg, B., Paul, W. E., and Bona, C. A. 1983. Idiotype-anti-idiotype regulation. IV. Expression of common regulatory idiotopes on fructosanbinding and non-fructosan-binding monoclonal immunoglobulin. J. Exp. Med. **158**: 515-528.

Greenwood, F. C., Hunter, W. M., and Glover, J. S. 1963. The preparation of ^{125}I-labelled human growth hormone of high specific radioactivity. Biochem. J. **89**: 114-123.

Grey, H. M., Mannik, M., and Kunkel, H. G. 1965. Individual antigenic specificity of myeloma proteins. Characteristics and localization to subunits. J. Exp. Med. **121**: 561-575.

Hammerling, U., and Westphal, O. 1967. Synthesis and use of o-stearoyl polysaccharides in passive hemagglutination and hemolysis. Eur. J. Biochem. **1**: 46-50.

Hannestad, K., Kao, M. S., and Eisen, H. N. 1972. Cell-bound myeloma proteins on the surface of myeloma cells: potential targets for the immune system. Proc. Natl. Acad. Sci. **69**: 2295-2299.

Hart, D., Wang, A. L., Pawlak, L., and Nisonoff, A. 1972. Suppression of idiotypic specificities in adult mice by administration of antiidiotypic antibody. J. Exp. Med. **135**: 1293-1300.

Heyman, B., Andrighetto, G., and Wigzell, H. 1982. Antigen-dependent IgM-mediated enhancement of the sheep erthyrocyte response in mice. Evidence for induction of B cells with specificities other than that of the injected antibodies. J. Exp. Med. **155**: 994-1009.

Hiernaux, J., and Bona, C. A. 1982. Shared idiotypes among monoclonal antibodies specific for different immunodominant sugars of lipopolysaccharide of different gram-negative bacteria. Proc. Natl. Acad. Sci. **79**: 1616-1620.

Hiernaux, J., Bona, C., and Baker, P. J. 1981. Neonatal treatment with low doses of anti-idiotypic antibody leads to the expression of a silent clone. J. Exp. Med. **153**: 1004-1008.

Inman, J. K. 1975. Thymus-independent antigens: the preparation of covalent, hapten-Ficoll conjugates. J. Immunol. **114**: 704-709.

Jerne, N. 1974. Towards a network theory of the immune system. Ann. Immunol. (Inst. Pasteur) **125C**: 373-389.

Jerne, N. K., and Nordin, A. A. 1963. Plaque formation in agar by single antibody-producing cells. Science **140**: 405.

Jerne, N. K., Roland, J., and Cazenave, P. A. 1982. Recurrent idiotopes and internal images. The Embo J. **1**: 243-247.

Keck, K. 1977. An easy method for labeling polysaccharides with radioactive iodine. Immunochem. **9**: 359-360.

Kelsoe, G., Reth, M., and Rajewsky, K. 1981. Control of idiotope expression by monoclonal anti-idiotope and idiotope-bearing antibody. Eur. J. Immunol. **11**: 418-423.

Kilbourne, E. D. 1978. Influenza as a problem in immunology. J. Immunol. **120**: 1447-1452.

Klinman, N. R., Pickard, A. R., Sigal, N. H., Gearhard, P. J., Metcal, E. S., and Pierce, S. K. 1976. Assessing B cell diversification by antigen receptor and precursor cell analysis. Ann. Immunol. (Inst. Pasteur) **127C**: 489-502.

Kotlarski, I., Courtenay, B. M., and Howard, J. G. 1973. Studies on immunological paralysis. XI. A comparison of the tolerogenicity in vitro of levan and type 3 pneumococcal polysaccharide. Eur. J. Immunol. **3**: 496-502.

Kunkel, H. G. 1970. Individual antigenic specificity, cross specificity and diversity of human antibodies. Fed. Proc. **29**: 55-58.

Kunkel, H. G., Agnello, V., Joslin, F. G., Winchester, R. J., and Capra, J. D. 1973. Cross-idiotypic specificity among monoclonal IgM proteins with anti-γ-globulin activity. J. Exp. Med. **137**: 331-342.

Kunkel, H. G., Mannik, M., and Williams, R. C. 1963. Individual antigenic specificity of isolated antibodies. Science **140**: 1218-1219.

Kunkel, H. G., Winchester, R. J., Joslin, F. G., and Capra, J. D. 1974. Similarities in the light chains of anti-γ-globulins showing cross-idiotypic specificities. J. Exp. Med. **139**: 128-136.

Legrain, P., Voegtle, D., Buttin, G., and Cazenave, P. A. 1981. Idiotype-anti-idiotype interactions and the control of the anti-β(2-6) polyfructosan response in the mouse: specificity and idiotypy of anti-ABPC48 anti-idiotypic monoclonal antibodies. Eur. J. Immunol. **11**: 678-685.

Lieberman, R., Bona, C., Chien, C. C., Stein, K. E., and Paul, W. E. 1979. Genetic and cellular regulation of the expression of specific antibody idiotypes in the anti-polyfructosan immune response. Ann. Immunol. (Inst. Pasteur) **130C**: 247-262.

Lieberman, R., Potter, M., Humphrey, W., and Chien, C. C. 1976. Idiotypes of inulin binding antibodies and myeloma proteins controlled by genes linked to the allotype locus of the mouse. J. Immunol. **117**: 2105-2111.

Lieberman, R., Potter, M., Humphrey, W., Mushinski, E. B., and Vrana, M. 1975. Multiple individual and cross-specific idiotypes on 13 levan-binding myeloma proteins of BALB/c mice. J. Exp. Med. **142**: 106-119.

Lieberman, R., Vrana, M., Humphrey, W., Chien, C. C., and Potter, M. 1977. Idiotypes of inulin binding myeloma proteins localized to variable region light and heavy chains: genetic significance. J. Exp. Med. **146**: 1294-1304.

Lindenmann, J. 1979. Homobodies: do they exist? Ann. Immunol. (Inst. Pasteur) **130C**: 311-318.

Lynch, R. G., Graff, R. J., Sirisinha, S., Simms, E. S., and Eisen, H. N. 1972. Myeloma proteins as tumor-specific transplantation antigens. Proc. Natl. Acad. Sci. **69**: 1540-1544.

McKean, D., Huppi, K., Bell, M., Staudt, L. M., Gerhard, W., and Weigert, M. 1984. Generation of antibody diversity in the immune response of BALB/c mice to influenza virus hemagglutinin. Proc. Natl. Acad. Sci. **81**: 3180-3184.

Marasco., W. A., and Becker, E. L. 1982. Anti-idiotype as antibody against the formyl peptide chemotaxis receptor of the neutrophil. J. Immunol. **128**: 963-968.

Meeker, T., Lowder, J., Cleary, M. L., Stewart, S., Warnke, R., Sklar, J., and Levy, R. 1985a. Emergence of idiotype variants during therapy of B cell lymphoma with anti-idiotype antibodies. N. Engl. J. Med. (in press).

Meeker, T. C., Lowder, J., Maloney, D. G., Miller, R. A., Thielmans, K., Warnke, R., and Levy, R. 1985b. A clinical trial of anti-idiotype therapy for B cell malignancy. Blood (in press).

Meinke, G. C., McConahey, P. J., and Spiegelber, H. L. 1974. Suppression of plasmacytoma growth in mice by immunization with myeloma proteins. Fed. Proc. **33**: 792.

Milburn, G. L., and Lynch, R. G. 1982. Immunoregulation of murine myeloma in vitro. II. Suppression of MOPC-315 immunoglobulin secretion and synthesis by idiotype-specific suppressor T cells. J. Exp. Med. **155**: 852-861.

Miller, R. A., Maloney, D. G., Warnke, R., and Levy, R. 1982. Treatment of B-cell lymphoma with monoclonal anti-idiotype antibody. N. Engl. J. Med. **306**: 517-522.

Miranda, J. J. 1972. Studies on immunological paralysis. IX. The immunogenicity and tolerogenicity of levan (polyfructose) in mice. Immunol. **23**: 829-842.

Nepom, J. T., Weiner, H. L., Dichter, M. A., Tardieu, M., Spriggs, D. R., Graum, C. F., Powers, M. L., Fields, B. N., and Greene, M. I. 1982. Identification of a hemagglutinin-specific idiotype associated with reovirus recognition shared by lymphoid and neural cells. J. Exp. Med. **155**: 155-167.

Nicolotti, R. A., Briles, D. E., Schroer, J. A., and Davie, J. M. 1980. Isoelectric focusing of immunoglobulins: improved methodology. J. Immunol. Methods **33**: 101-115.

Ollo, R., Auffray, C., Sikorav, J. L., and Rougeon, F. 1981. Mouse heavy chain variable regions: nucleotide sequence of a germ-line V_H segment. Nucl. Acids Res. **9**: 4099-4109.

Oudin, J., and Cazenave, P. A. 1971. Similar idiotypic specificities in immunoglobulin fractions with different antibody functions or even without detectable antibody function. Proc. Natl. Acad. Sci. **68**: 2616-2620.

Oudin, J., and Michel, M. 1963. Une nouvelle forme d'allotypie des globulines y du serum de lapin, apparement liee a la function et a la specificite anticorps. C.R. Acad. Sci. Paris **257**: 805-808.

Oudin, J., and Michel, M. 1969a. Idiotypy of rabbit antibodies. I. Comparison of idiotypy of antibodies against Salmonella typhi with that of antibodies against other bacteria in the same rabbits, or of antibodies against Salmonella typhi in various rabbits. J. Exp. Med. **130**: 595-617.

Oudin, J., Michel, M. 1969b. Idiotypy of rabbit antibodies. II. Comparison of idiotypy of various kinds of antibodies formed in the same rabbits against Salmonella typhi. J. Exp. Med. **130**: 619-642.

Paraf, A., Guerin-Marchand, C., Aubry, J., and Dubert, J. M. 1975. Inhibition of murine plasmacytoma TEPC15 by its specific antigen. Nature **255**: 488-489.

Paul, W. E., and Bona, C. 1982. Regulatory idiotypes and immune networks: a hypothesis. Immunol. Today **3**: 230-234.

Pfaffenbach, G., Lamm, M. E., and Gigli, I. 1982. Activation of the guinea pig alternative complement pathway by mouse IgA immune complexes. J. Exp. Med. **155**: 231-247.

Potter, M. 1977. Antigen-binding myeloma proteins of mice. Adv. Immunol. **25**: 141-211.

Potter, M., and Lieberman, R. 1970. Common individual antigenic determinants in five of eight BALB/c IgA myeloma proteins that bind phosphorylcholine. J. Exp. Med. **132**: 737-751.

Reth, M., Kelsoe, G., and Rajewsky, K. 1981. Idiotypic regulation by isologous monoclonal anti-idiotype antibodies. Nature **290**: 257-259.

Rittenberg, M. B., and Pratt, K. L. 1969. Anti-trinitrophenyl (TNP) plaque assay. Primary response of BALB/c mice to soluble and particulate immunogen. Proc. Soc. Exp. Biol. Med. **132**: 575-581.

Rohrer, J. W., and Lynch, R. G. 1977. Specific, immunologic regulation of differentiation of immunoglobulin expression in MOPC315 cells during in vivo growth in diffusion chambers. J. Immunol. **119**: 2045-2053.

Rohrer, J. W., Odermatt, B., and Lynch, R. G. 1979. Immunoregulation of murine myeloma: isologous immunization with M315 induces idiotype-specific T cells that suppress IgA secretion by MOPC-315 cells in vivo. J. Immunol. **122**: 2011-2019.

Rohrer, J. W., Vasa, K., and Lynch, R. G. 1977. Myeloma cell immunoglobulin expression during in vivo growth in diffusion chambers: evidence for repetitive cycles of differentiation. J. Immunol. **119**: 861-866.

Schreiber, A. B., Couraud, P. O., Andre, C., Vray, B., and Strosberg, A. D. 1980. Anti-alprenolol anti-idiotypic antibodies bind to β-adrenergic receptors and modulate catecholamine-sensitive adenylate cyclase. Proc. Natl. Acad. Sci. **77**: 7385-7389.

Sege, K., and Peterson, P. A. 1978a. Use of anti-idiotypic antibodies as cell-surface receptor probes. Proc. Natl. Acad. Sci. **75**: 2443-2447.

Sege, K., and Peterson, P. A. 1978b. Anti-idiotypic antibodies against anti-vitamin A transporting protein react with prealbumin. Nature **271**: 167-168.

Shulman, M., Wilde, C. D., and Kohler, G. 1978. A better cell line for making hybridomas secreting specific antibodies. Nature **276**: 269-270.

Sirisinha, S., and Eisen, H. N. 1971. Autoimmune antibodies to the ligand binding sites of myeloma proteins. Proc. Natl. Acad. Sci. **68**: 3130-3135.

Slater, R. J., Ward, S. M., and Kunkel, H. G. 1955. Immunological relationships among the myeloma proteins. J. Exp. Med. **101**: 85-108.

Snodgrass, H. R., Bosma, M. J., and Wilson, D. B. 1981. T lymphocytes specific for Ig allotype. II. Cloned Igh-1^b specific cytotoxic T cells. J. Exp. Med. **154**: 491-500.

Spring-Stewart, S., and Nisonoff, A. 1973. Effect of blocking the active site of an antibody molecule on the expression of its idiotypic determinants during immunization. J. Immunol. **110**: 679-684.

Stein, K. E., Bona, C., Lieberman, R., Chien, C. C., and Paul, W. E. 1980. Regulation of the anti-inulin antibody response by a nonallotype-linked gene. J. Exp. Med. **151**: 1088-1102.

Victor-Kobrin, C., Bonilla, F. A., Bellon, B., and Bona, C. A. 1985. Immunochemical and molecular characterization of regulatory idiotopes expressed by monoclonal antibodies exhibiting or lacking β2-6 fructosan binding activity. J. Exp. Med. **162**: (in press)

Vrana, M., Tomasic, J., and Glaudemans, C. P. J. 1976. Purification of homogeneous murine immunoglobulins with anti-fructofuranan specificity. J. Immunol. **116**: 1662-1663.

Wikler, M., Demeur, C., Dewasme, G., and Urbain, J. 1980. Immunoregulatory role of maternal idiotypes. Ontogeny of immune networks. J. Exp. Med. **152**: 1024-1035.

Wikler, M., Franssen, J. D., Collignon, C., Leo, O., Mariame, B., Van de Walle, P., DeGroote, D., and Urbain, J. 1979. Idiotypic regulation of the immune system. Common idiotypic specificities between idiotypes and antibodies raised against anti-idiotypic antibodies in rabbits. J. Exp. Med. **150**: 184-195.

Wilder, R. L., Yuen, C. C., Subbarao, B., Woods, V. L., Alexander, C. B., and Mage, R. G. 1979. Tritium (^3H) radiolabeling of protein A and antibody to high specific activity. Application

to cell surface antigen radioimmunoassays. J. Immunol. Methods **28**: 255-266.

Williamson, A. R., Zitron, I. M., and McMichael, A. J. 1976. Clones of B lymphocytes: their natural selection and expansion. Fed. Proc. **35**: 2195-2201.

Yelton, D. E., and Scharff, M. D. 1982. Mutant monoclonal antibodies with alterations in biological functions. J. Exp. Med. **156**: 1131-1148.