IDIOTYPIC REPERTOIRE OF ANTI-HEN EGGWHITE LYSOZYME ANTIBODIES PROBED WITH HYBRIDOMAS

Selection after Immunization of an IdX Marker Common to Antibodies of Distinct Epitope Specificity*

BY DENNIS W. METZGER,‡ ABRAHAM FURMAN, ALEXANDER MILLER, AND ELI E. SERCARZ

From the Department of Microbiology, University of California, Los Angeles, California 90024

The immune response to a specific antigen can frequently be shown to be dominated by antibody molecules bearing a particular idiotype (IdX)[1] (1). Although an idiotype is defined as a variable-region marker and is usually associated with a particular antigen specificity, several groups (2–13) have shown that individual idiotypic determinants can sometimes be found on antibodies of distinct specificities. This finding, together with the evidence demonstrating IdX-specific helper (14, 15) and suppressor T cells (16, 17), is in accord with Jerne's network theory (18) and suggests that idiotypic recognition per se plays a pivotal role in regulating IdX expression. Nevertheless, little is known about the control of IdX expression after antigen injection and in only a few cases has it been shown that idiotypic modulation actually occurs during the specific antibody response (19–21).

In every mouse strain examined, most antibody produced against the small protein antigen, hen eggwhite lysozyme (HEL), appears to be specific for a restricted portion of the HEL molecule and bears a predominant idiotype (IdX-HEL) (22, 23). IdX-HEL can be defined by sera raised in guinea pigs or rabbits against conventional anti-HEL antibody; using monoclonal reagents, we recently demonstrated that the presence of IdX-HEL can be dissociated from the major epitypic specificity of anti-HEL activity (8). In this paper, we present evidence that IdX-HEL-bearing antibody becomes selected for during the maturation of the anti-HEL response. Thus, epitope-specific and idiotype-specific recognition processes are coordinated in the regulation of the response to HEL.

* Supported in part by grant IM-263 from the American Cancer Society and grants CA-24442 and AI-11183 from the U. S. Public Health Services.

‡ Recipient of postdoctural traineeship NRSA-NCI 5 T32 CA09120-05 from the National Cancer Institute. Present address: Department of Immunology, St. Jude Children's Research Hospital, Memphis, Tenn. 38101.

[1] *Abbreviations used in this paper:* AgTh, antigen-specific T helper cell; CFA, complete Freund's adjuvant; FCS, fetal calf serum; HAT, hypoxanthine, aminopterin, and thymidine medium; HEL, hen eggwhite lysozyme; HUL, human lysozyme; IdX, predominant idiotype; IdXTh, T helper cell specific for IdX; IEF, isoelectric focusing; N-C, NH_2-terminal-COOH-terminal peptide; P3, P3X63Ag8 myeloma cell line; PFC, plaque-forming cells; RBC, erythrocytes; S194/5, myeloma cell line S194/5.XX0.BU.1; Sp2/0, myeloma cell line Sp2/0-Ag14.

Materials and Methods

Animals and Cell Lines. 6–10-wk-old B10.A and A/J mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. (BALB/c × B10.A)F_1 and (BALB/c × A/J)F_1 mice were bred in our colony. The cell lines P3 × 63Ag8 (P3) (24), S194/5.XXO.BU.1 (S194/5) (25), and Sp2/O-Ag14 (Sp2/0) (26) were obtained from The Salk Institute, La Jolla, Calif. The P3 line secretes IgG1 MOPC21 immunoglobulin; the S194/5 and Sp2/O lines are variants that have lost the ability to synthesize immunoglobulin. The cell lines were maintained in RPMI 1640 (Grand Island Biological Co., Grand Island, N. Y.) containing 15% fetal calf serum (FCS) (Microbiological Associates, Walkersville, Md.), 2 mM L-glutamine, 1 mM sodium pyruvate, 100 U/ml penicillin, and 100 μg/ml streptomycin (all from Grand Island Biological Co.).

Cell Fusion. Mice were immunized intraperitoneally, subcutaneously, and in each footpad with 100 μg, 25 μg, and 25 μg, respectively, of HEL (Societa Prodotti Antibiotici, Milan, Italy), emulsified in complete Freund's adjuvant (CFA) (Difco Laboratories, Detroit, Mich.). For a primary response, cell suspensions were prepared from combined parathymic, periaortic, inguinal, and popliteal lymph nodes, removed 9 d after immunization. For a secondary response, the primed mice were boosted intraperitoneally, subcutaneously, and in the footpad with 100 μg, 25 μg, and 25 μg, respectively, of HEL in incomplete Freund's adjuvant 28 d after the initial immunization. 3–5 d later, cell suspensions were prepared from the spleens and lymph nodes. The lymphoid cells (1×10^7) were fused with 1×10^8 myeloma cells according to the method of Gefter et al. (27) using 35% polyethylene glycol 1540 (J. T. Baker Chemical Co., Phillipsburg, N. J.). After fusion, the cultures were gradually selected in microtiter plates (Flow Laboratories, Hamden, Conn.) over a 2-wk period in medium containing hypoxanthine, aminopterin, and thymidine (HAT medium) and then incubated an additional 2 wk in HAT medium without aminopterin before transfer to RPMI 1640–15% FCS. Under these conditions, ~10% of the wells showed cell growth 2–4 wk after fusion, and of these, 30–50% contained anti-HEL antibody.

Screening the Hybridoma Cultures for Anti-HEL Antibody. Cells that grew in HAT medium were assayed for secretion of anti-HEL antibody using anti-mouse immunoglobulin facilitated hemagglutination of HEL-coupled sheep erythrocytes (RBC). HEL was coupled to RBC by the mannitol-carbodiimide method previously described by our laboratory (28). The test was performed in U-bottomed microtiter plates (Cooke Laboratory Products, Div. Dynatech Laboratories, Inc., Alexandria, Va.) using 25 μl of culture supernate and 25 μl of 0.1% HEL-RBC suspended in a 1:75 dilution of guinea pig anti-mouse immunoglobulin serum. This technique is capable of detecting <1 ng of anti-HEL antibody.

Cloning and In Vivo Propagation of Anti-HEL Hybridomas. Hybridoma cultures that secreted anti-HEL antibody were cloned by limiting dilution. The cloning was performed in microtiter plates (Flow Laboratories) using normal BALB/c spleen cells (3×10^5 cells/well) as a feeder layer. The efficiency of cloning was confirmed by isoelectric focusing (IEF). For ascites production, 1×10^6–5×10^6 cloned cells were injected intraperitoneally into F_1 (BALB/c × B10.A; BALB/c × A/J) mice that had been injected 1–2 wk earlier with 0.5 ml Pristane (2,6,10,14-tetramethylpentadecane) (Aldrich Chemical Co., Milwaukee, Wisc.). Ascites fluid usually developed after 2 wk and yielded on the average 5 mg/ml specific antibody.

Nomenclature for Anti-HEL Hybridomas. The nomenclature adopted for the various hybridomas is as follows: the first number represents the myeloma cell parent: a "2" signifies the Sp2/0 myeloma, a "3" signifies the P3 myeloma, and a "5" indicates the S194/5 myeloma. The next two numbers represent when the lymphoid cells were obtained from the immunized donor, e.g., "19" indicates that fusion was performed on a primary response day 9 sample (an "early" hybridoma) and "25" indicates that fusion was performed on day 5 of the secondary response (a "late" hybridoma). The final three symbols identify the particular cell line. The mouse strain used as the source of lymphoid cells has not been denoted because we have not observed any qualitative differences in the pattern of idiotype development in A/J and B10.A mice to HEL.

Preparation of Anti-Idiotype Antisera. Anti-HEL antibody from individual B10.A mice was obtained from ascites fluids that were raised by the procedure of Tung and Nisonoff (29) using multiple injections of HEL-CFA at a 1:9 ratio. The immunoglobin fraction of the ascites was passed over an Affigel-10 immunoadsorbent column (Bio-Rad Laboratories, Richmond, Calif.)

coupled with HEL, and the bound antibody was eluted with 0.2 M glycine sulfate buffer, pH 2.3, neutralized with 1.0 M Tris buffer, and dialyzed extensively against physiological saline.

Anti-idiotype antisera were raised in guinea pigs and rabbits by priming with 200 μg of the isolated anti-HEL antibody in CFA, followed by boosting 4 wk later with 200 μg in saline. Each anti-idiotype antiserum was passed over an HEL immunoadsorbent and then isotypic and allotypic specificities were removed by repeated absorption over an Affigel-10 column to which normal B10.A immunoglobulin had been conjugated.

Anti-Idiotype Absorption of IEF Patterns. IEF of anti-HEL ascites fluids was performed in 5% polyacrylamide gels as described by Awdeh et al. (30) as modified in our laboratory by Cecka et al. (31). The focused gels were exposed to ^{125}I-HEL at 37°C for 15 min. The gels were then incubated in 18% (wt:vol) sodium sulfate to precipitate the immunoglobulin, fixed in 0.2% (vol:vol) glutaraldehyde, washed, dried, and exposed on Kodak NS-2T x-ray film (Eastman Kodak Co., Rochester, N. Y.).

For absorption of IEF patterns, various amounts of anti-idiotype antisera were mixed with a constant amount of the ascites fluid to be tested and the mixtures were then incubated at 37°C for 30 min and at 4°C overnight. The entire contents were plated on IEF polyacrylamide gels next to control mixtures containing an equivalent amount of ascites fluid that had been incubated with saline. The idiotype-anti-idiotype complexes that form during the incubation period are apparently too large to enter the gel, leading to the elimination of reactive bands.

Anti-Idiotype Inhibition of Antigen Binding. For inhibition of antigen binding by anti-idiotype, 20-μl samples of anti-HEL ascites fluid or serum at a dilution that binds 30–80% of 10 ng ^{125}I-HEL were incubated with varying amounts of anti-idiotype antiserum at 37°C for 60 min. To these mixtures was added 100 μl of 100 ng/ml ^{125}I-HEL and incubation was continued at 37°C for 30 min and at 4°C overnight. All dilutions were made in phosphate-buffered saline containing 20% normal rabbit serum. The amount of bound antigen was determined as described previously (23).

Anti-Idiotype Inhibition of Plaque-forming Cells (PFC). Anti-HEL IgG plaque inhibition was performed in Cunningham slide chambers (32) using an anti-idiotype antiserum that was absorbed extensively with mouse RBC and HEL-coupled goat RBC before use. Immunized cell populations were adjusted to yield ~200 PFC/slide. Goat RBC and lysozyme-coupled goat RBC were used at a 1% final concentration. Various dilutions of anti-idiotype were added to the cells, together with guinea pig complement and guinea pig anti-mouse immunoglobulin facilitating serum, to a total volume of 200 μl. The mixtures were incubated in slide chambers for 2 h at 37°C and the PFC were enumerated immediately.

Absorption of Anti-Idiotype with Hybridoma Antibodies. Normal A/J immunoglobulin eluted from a protein A column or hybridoma antibodies eluted from an HEL column were trace-labeled with ^{125}I and coupled to Affigel-10 beads. The anti-idiotype antiserum (250 μl) was then incubated with 50 μg insolubilized protein at 4°C overnight with continuous rocking. The mixtures were centrifuged and the supernates were tested for inhibition of ^{125}I-HEL binding to an HEL-induced A/J ascites fluid.

Results

Derivation and Analysis of Anti-HEL Hybridomas. A preliminary hybridization experiment with lymphocytes from singly injected mice had yielded hybrid cell lines that in every case failed to display the predominant anti-HEL idiotype. To explore the reason for this apparent anomaly, several hybridizations were performed by fusing P3, SP2/O, and S194/5 myeloma cells with both primary and secondary response lymphocytes. Lymphoid cells for the production of "early" hybridomas were obtained from B10.A or A/J mice 9 d after priming, i.e., at the peak of the primary anti-HEL response. "Late" hybridomas were derived using cells from primed and boosted mice undergoing a secondary response to HEL on day 3 or 5 after boosting. Over 40 anti-HEL hybridomas were established by limiting dilution cloning and representative lines were grown as ascites fluids in syngeneic mice.

The heavy and light chain composition of the anti-HEL monoclonal antibodies

was determined by a solid-phase radioimmunoassay using rabbit anti-subclass antisera (kindly provided by Dr. J. Teale and Dr. N. Klinman, Scripps Clinic and Research Foundation, La Jolla, Calif.) and ^{125}I-goat anti-rabbit IgG to assess binding of the hybridoma culture supernates to HEL. It was found that all but two of the lines secreted IgG1-κ antibody; one line (525.6D7) produced IgG2b antibody, whereas the other (525.5E5) produced IgMκ antibody (data not shown). These results agree with analyses of both primary and secondary response sera from HEL-immune mice in which almost all of the antibody appears to be IgG1κ and only very little IgM antibody is detected (A. Furman, unpublished data).

Idiotypic Characterization of Hybridoma Antibodies. Our laboratory has previously shown (15, 16) that anti-idiotypic antisera raised against restricted anti-HEL serum antibody populations recognize almost all anti-HEL activity produced in vivo by several strains of primed and boosted mice. It was therefore of interest to determine whether such anti-idiotypic antisera would react with the hybridoma antibodies.

Absorption of antibody IEF patterns by anti-idiotype was used for initial idiotypic screening (23). In this assay, removal of idiotype-positive bands by formation of idiotype-anti-idiotype complexes, which are apparently too large to enter the gel, results in the elimination of reactive bands from the IEF pattern. An anti-idiotype antiserum raised against isolated anti-HEL antibody from a hyperimmunized B10.A mouse was mixed with ascitic fluids containing monoclonal antibody. The mixtures were plated on IEF gels next to ascitic fluids incubated with saline as a control. This treatment removes the entire anti-HEL banding pattern from antibodies of HEL-hyperimmunized mice, but has little effect on murine anti-human lysozyme (HUL) antibody, which is only poorly cross-reactive with HEL (23). Similarly, representative late hybridoma antibodies derived from primed and boosted mice are, in every case, totally reactive with the anti-idiotype antiserum (Fig. 1). This finding was obtained even with monoclonal antibodies of different fine specificities, e.g., 525.6C10 and

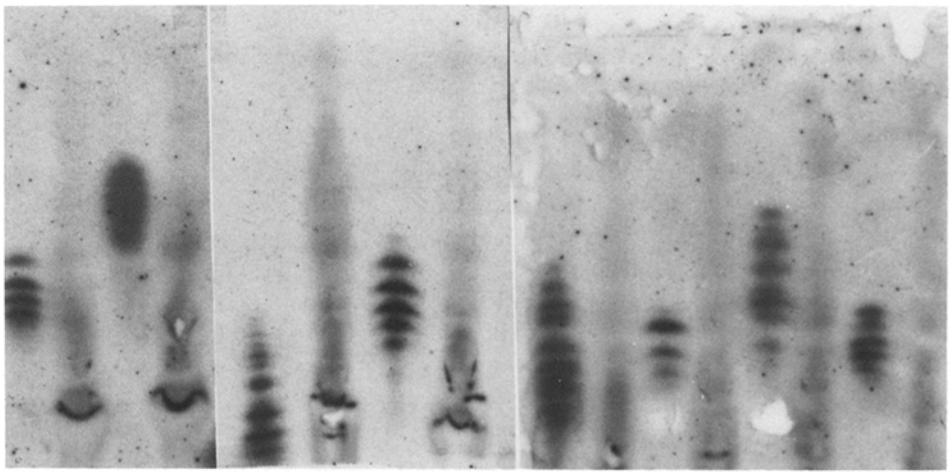

FIG. 1. Reaction of anti-idiotype with late hybridoma antibodies. Hybridoma anti-HEL ascitic fluids were incubated with saline (a, c, e, g, i, k, m, o) or anti-idiotype (b, d, f, h, j, l, n, p) at a 1:9 ratio before focusing. The gels were incubated with ^{125}I-HEL.

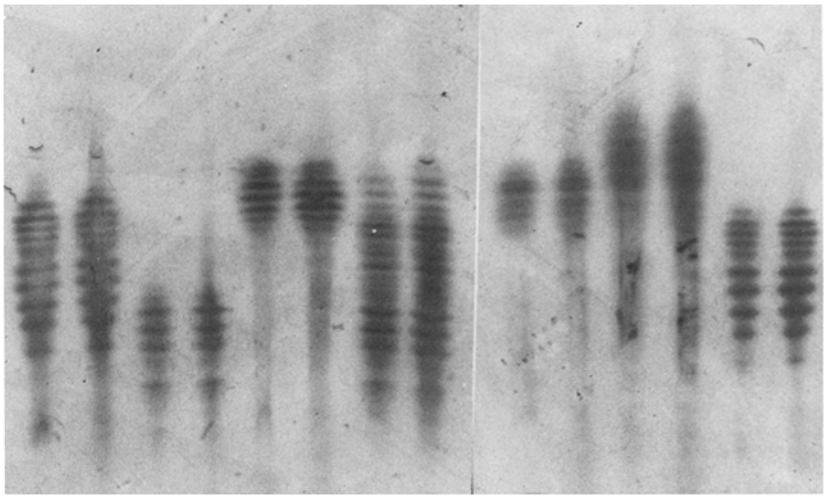

FIG. 2. Failure of anti-idiotype to react with early hybridoma antibodies. Hybridoma anti-HEL ascitic fluids were incubated with saline (a, c, e, g, i, k, m) or anti-idiotype (b, d, f, h, j, l, n) at a 1:9 ratio before focusing. The gels were incubated with ^{125}I-HEL.

225.2D1,[2] and peptide specificities, e.g., 525.5E4 and 525.6D7 (8). Thus, as shown in ref. 8, antibodies specific for distinct epitopes on the HEL molecule are reactive with anti-idiotype antibody.

Early hybridoma antibodies derived from mice undergoing a primary response to HEL were tested in a similar manner for idiotypy. In this case, however, we found that the IEF banding patterns of early monoclonal antibodies obtained from either A/J or B10.A mice were unaffected by anti-idiotype treatment (Fig. 2).

This intriguing result indicated a difference between the idiotypy of early and late hybridoma antibodies. This was confirmed by using the fact that anti-idiotype, but not polyvalent anti-immunoglobulin antiserum, interferes with the binding of anti-HEL antibody to ^{125}I-HEL in a standard Farr-type assay. Once again, it was observed that anti-idiotype inhibits the binding of late hybridoma antibodies to HEL but has little effect on early antibodies (Fig. 3). This experiment further shows that anti-idiotype competes equally well with the binding of HEL to antibodies of distinct peptide specificity.

Individual Mice Express the Predominant Idiotype Only after Prolonged Exposure to HEL. The findings with hybridoma antibodies indicated that the predominant idiotypic marker(s) becomes expressed only after maturation of the anti-HEL response. It was therefore of interest to determine whether parallel results would be obtained with serum anti-HEL antibody produced in vivo by individual mice. To test this possibility, anti-idiotype was used to inhibit PFC formation by parathymic lymph node cells that were obtained from B10.A mice undergoing primary and secondary responses to HEL. As seen in Fig. 4, secondary, but not primary PFC were totally inhibited by anti-idiotype antiserum. Anti-HUL PFC from HUL-immunized B10.A mice were almost unaffected by this treatment.

[2] Metzger, D. W., L.-K. Ch'ng, A. Miller, and E. E. Sercarz. The lysozyme-specific repertoire: I. Specificity repertoire of anti-hen eggwhite lysozyme antibodies probed with hybridomas. Manuscript in preparation.

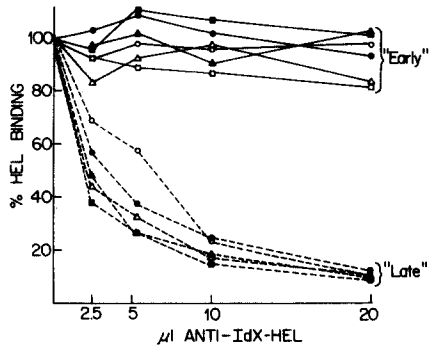

FIG. 3. Anti-idiotype inhibition of HEL binding to late but not early hybridoma antibodies. Effects of increasing amounts of anti-idiotype on early (——) and late (---) hybridoma anti-HEL binding to ^{125}I-HEL. Early: (●), 319.AG5; (○), 319.AD2; (▲), 519.2E6; (△), 519.2E5; (■), 519.2F4; (□), 219.1G9. Late: (●), 525.5E4; (○), 525.6D7; (▲), 325.1G11; (△), 225.2D5; (■), 225.2D1.

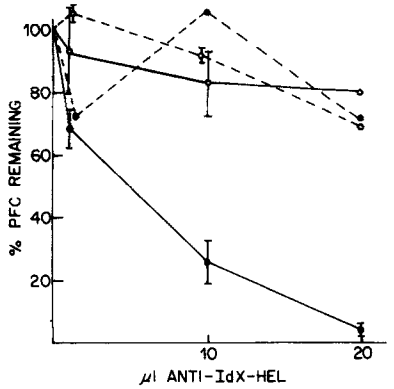

FIG. 4. Anti-idiotype inhibition of secondary but not primary response anti-HEL PFC formation. Effects of increasing amounts of anti-idiotype on primary day 9 (○) and secondary day 6 (●) PFC formation by parathymic lymph nodes from HEL- (——) or HUL- (---) immunized B10.A mice.

These results were confirmed by following the anti-HEL sera of individual mice for the appearance of the anti-HEL isotypic marker. B10.A and A/J mice were primed with HEL and bled 9 d later. The mice were rested for 3 wk, boosted, and bled 5 d after secondary immunization. These primary and secondary response sera were then tested for anti-idiotype-mediated inhibition of HEL binding. It was found that, in every animal, secondary response serum antibody was almost completely inhibited by anti-idiotype, whereas primary response antibody was not inhibited (Fig. 5). Anti-idiotype had no effect on the binding of ^{125}I-HUL to anti-HUL antibody. Experiments designed to assess peptide specificity in a solid-phase radioimmunoassay have shown approximately the same relative proportions of antibody reactive with the NH_2-terminal-COOH-terminal (N-C) peptide (amino acids 1–17; cys 6–cys 127; 120–129) and the mixed-disulfide L_{II} peptide (amino acids 13–105) of HEL in both primary and secondary response sera (data not shown). Thus, a predominant marker(s) that is unrelated to epitope specificity becomes selected for during the course of the immune response of HEL.

Anti-Idiotype Induced with a Heterogenous Antibody Population Recognizes Primarily a Major

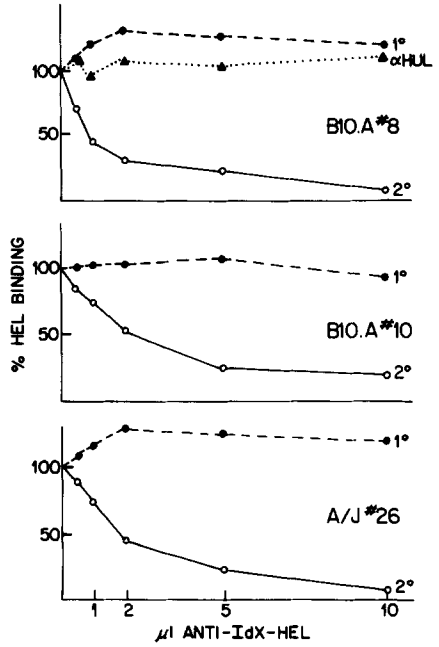

FIG. 5. Serum anti-HEL antibody in individual mice displays the IdX-HEL specificity only late in the immune response. Effects of increasing amounts of anti-idiotype on B10.A and A/J primary day 9 and secondary day 5 serum anti-HEL binding to ^{125}I-HEL. Primary day 9 anti-HEL, (●), secondary day 5 anti-HEL, (○). The effect of anti-idiotype on the binding of anti-HUL to ^{125}I-HUL is also shown (▲) in B10.A #8, immunized with both antigens.

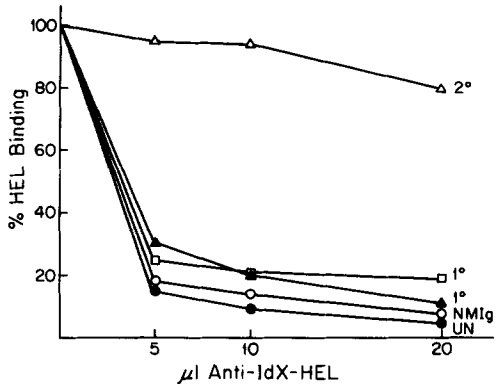

FIG. 6. Attempted absorption of the anti-IdX activity from guinea pig antiserum raised against serum idiotype was performed with 50 μg of three different affinity-purified monoclonal antibodies, or normal immunoglobulin 5E4 late, (△); AG5, (□); and AD-2 early, (▲); unabsorbed, (●); normal mouse immunoglobulin absorbed, (○).

Idiotypic Determinant (IdX-HEL). Because the anti-idiotypic antisera used in this study were raised against a heterogenous antibody population obtained from a hyperimmunized mouse, it is unclear whether these reagents were recognizing several distinct idiotypes or a major idiotypic determinant, IdX-HEL, shared by almost all secondary anti-HEL antibody. To answer this question, anti-idiotype was absorbed with each of several hybridoma antibodies and subsequently tested for residual activity against a

heterogenous, HEL-induced antibody population. We reasoned that if the anti-idiotype were recognizing many different idiotypic markers, absorption with monoclonal antibody would have little or no effect on its ability to react with heterogenous antibody. However, if one set of common markers was being recognized primarily, this absorption should completely abolish reactivity. An anti-idiotype antiserum was absorbed with 50 μg purified normal mouse immunoglobin or various hybridoma antibodies coupled to Affigel beads. The absorbed antiserum was then assessed for inhibition of HEL binding to a heterogenous anti-HEL ascites fluid. It was found that absorption with normal immunoglobin or early hybridoma antibodies had little effect on anti-idiotype activity compared with an unabsorbed control, whereas absorption with a single late hybridoma antibody completely abolished activity (Fig. 6). Absorption with the immunoglobulin fractions of two other late hybridoma ascitic fluids also removed all of the activity from this anti-idiotype antiserum (data not shown). Thus, the anti-idiotype antisera appear to recognize an IdX-HEL marker common to all late hybridoma and serum anti-HEL but absent on early hybridoma and serum antibody.

Discussion

We have used both hybridoma and serum antibodies and PFC to analyze the changing idiotypic nature of the antibody produced in response to a small protein antigen, HEL. Results with the two mouse strains studied here indicate that a predominant idiotypic marker, IdX-HEL, is found on almost all secondary response anti-HEL antibody in each mouse (Fig. 5). Yet IdX-HEL is not expressed on primary response antibody, IdX-HEL gaining predominance only after development of the anti-HEL response. This is indicative of a selective pressure related to the maturational stage of the response but not directly related to antigen specificity.

Almost all secondary response anti-HEL antibody derived from hybridomas or produced in individual animals expressed the predominant IdX-HEL marker. Similarly, secondary PFC were totally inhibited by anti-idiotype antisera, whereas primary PFC were not affected. The absorption studies with monoclonal antibodies confirm the previous conclusion (23) that IdX-HEL is a determinant shared by all idiotype-positive serum antibody rather than a number of different idiotypic markers carried by distinct B cell populations. This interpretation is supported by the finding that anti-idiotypic antisera raised against several single anti-HEL hybridomas recognize >90% of the anti-HEL antibody in the sera of primed and boosted mice (33).

In agreement with our previous results using anti-idiotypic reagents raised against hybridoma antibodies (8), IdX-HEL was found on late hybridoma antibodies of distinct epitope specificity. Experiments using serum antibody similarly indicated that idiotype was unrelated to specificity. Nevertheless, anti-idiotypic antibody was still able to inhibit the binding to HEL. Probably IdX-HEL is a variable-region framework determinant close to the antigen-combining site for the bulky macromolecular ligand. Although most studies involving myeloma proteins and antibody responses in inbred mice have indicated that idiotypy is closely related to antigen specificity, our findings, like those of Oudin and Michel (2), Cazenave (3), and others (4–7, 9–13), clearly show that idiotypy can in some instances be dissociated from specificity. In particular, Eichmann et al. (4) have shown that the A5A idiotype can occur on immunoglobulin molecules of A/J mice that do not bind streptococcal A

carbohydrate. Also working with mice, Enghofer et al. (6) have shown that immunoglobulins with specificities for different carbohydrates can share idiotypy. More recently, experiments exhibiting dissociation of idiotype from antigen specificity have been reported for the cross-reactive idiotype associated with anti-azophenylarsonate binding (11), the 460 idiotype associated with anti-trinitrophenyl binding (12), the GTGL idiotype (9), the T15 idiotype (13), and idiotype associated with anti-myoglobin antibodies (10). Possibly all "predominant IdX idiotype" systems involve regulatory idiotopes that are not confined to antibodies of a single specificity. Of course, idiotopes unique to single hybridomas or subgroups of hybridomas can also be found.

Because IdX-HEL is not associated with epitope specificity, antigen-specific cells evidently do not play a direct role in the later predominance of this idiotype. Instead, idiotype selection presumably mediated by idiotype-specific T helper cells (IdXTh) can be invoked as the major causative agent for this selection; recent evidence has shown that such IdXTh cells are needed together with antigen-specific T helper cells (AgTh) for the optimal induction of an in vitro anti-HEL antibody response (15). Others have similarly demonstrated the importance of idiotype- (14), allotype- (34), and isotype-specific (35) helper T cells in other antigen systems.

Perhaps most interesting and in marked contrast to secondary response anti-HEL activity, the IdX-HEL marker was not detectable on antibody obtained shortly after HEL immunization or on the early hybridomas. It is not clear at present whether an early, alternative IdX exists or whether there is simply a broad diversity of clonotypes with individual idiotypic markers. Preliminary evidence using anti-hybridoma idiotypic antisera suggests that some early hybridoma antibodies share one or more determinants with late hybridomas. Idiotope analysis is underway in this and other laboratories (36, 37) to reveal the complexity of the anti-HEL idiotypic system.

The interactions that lead to the predominance of a singular idiotypic motif need to be defined clearly. One of the ambiguous areas in the study of idiotypic systems is the kinetics of expression of the predominant idiotype. The impression can be gleaned from several systems (e.g., 19–21) that IdX is expressed early on a majority of antibody molecules, and subsequently its predominance abates, owing to replacement or conjunction with other molecular species. In contrast, the anti-lysozyme system is characterized by a clear-cut lag in IdX appearance; a detailed examination of IdX-HEL expression will be published elsewhere.

It is possible that in certain strains, whose idiotype-specific T cells have been primed by self-constituents, IdX predominance is maintained by continual environmental antigenic challenge. This would establish a regulatory circuitry that would be engaged after antigen administration to promote the early appearance of IdX and its subsequent suppression. It is interesting that in the anti-HEL response, the forces influencing IdX-HEL predominance are especially powerful, because once established, IdX-HEL seems to maintain dominance.

In summary, we conclude that two different selective pressures act independently in determining the nature of the antibody response to HEL. Because it has been found that AgTh cells of H-2^a mice primarily recognize a determinant(s) in the L_{II} region of HEL (38), under the influence of the H-2 complex, we would like to attribute the prevalent epitypic specificity of the B cells, i.e., N-C specificity, to antigen-bridging constraints in T-B cell collaboration, which require the presentation of an opposed epitope to the B cell. If antigen-specific selection occurs early, with little involvement

of IdXTh, then antibody produced a short time after HEL immunization would bear a heterogenous array of idiotypic markers. At this point, those N-C-specific and rare L_{II}-specific B cell clones displaying IdX-HEL would be selected by IdXTh cells for further expansion. Thus, a combination of potent idiotypic selection by IdXTh cells and epitope selection by AgTh cells would explain the common finding of convergence between idiotypy and antigen specificity. We have not as yet detected IdX-HEL-displaying antibody among early populations, possibly because of the relatively small sample size of our early hybridomas and the inability of our serum analyses to detect minor proportions of IdX-HEL-positive antibody.

It remains a major puzzle why the IdX-HEL should be so prevalent and dominant not only in every strain of mouse but also in deer mice and rabbits (23; C. Benjamin, unpublished observations). Further work is also needed to determine how the IdXTh cells become activated, although we have discussed several possibilities elsewhere (39).

Summary

A panel of hybridoma antibodies obtained from lymphoid cells that were fused during a primary response ("early") or a secondary response ("late") gave results concordant with analysis of conventional, in vivo-produced anti-lysozyme idiotypes: early antibodies did not display the predominant anti-hen eggwhite lysozyme idiotype (IdX-HEL), whereas late antibodies all displayed IdX-HEL. Furthermore, individual late hybridomas could each remove the entire anti-IdX-HEL activity by absorption, whereas early hybridomas could not. The epitope specificities of the hybrodomas in both the early and late populations were heterogenous. We conclude that epitypic specificity in the response to HEL is determined independently from idiotypic specificity and that the predominant idiotype is selected for during the maturation of the anti-lysozyme response.

Received for publication 5 May 1981.

References

1. Eichmann, K. 1978. Expression and function of idiotypes on lymphocytes. *Adv. Immunol.* **26:** 195.
2. Oudin, J., and Michel, M. 1969. Idiotypy of rabbit idiotypes. II. Comparison of idiotypy of various kinds of antibodies formed in the same rabbits against *Salmonella typhi*. *J. Exp. Med.* **130:**619.
3. Cazenave, P.-A. 1973. L'idiotypie comparee des anticorps qui, dans le serum d'un lapin immunise contre la serumalbumine humaine, sont diriges contre des regions differente de cet antigene. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **31:**348.
4. Eichmann, K., A. Coutinho, and F. Melchers. 1977. Absolute frequencies of lipopolysaccharide-reactive B cells producing A5A idiotype in unprimed, streptococcal A carbohydrate-primed, anti-A5A idiotype-sensitized and anti-A5A idiotype-suppressed A/J mice. *J. Exp. Med.* **146:**1436.
5. Karol, R., M. Reichlin, and R. W. Noble. 1978. Idiotypic cross-reactivity between antibodies of different specificities. *J. Exp. Med.* **148:**1488.
6. Enghofer, E. M., C. P. J. Glaudemans, and M. J. Bosma. 1979. Immunoglobulins with different specificities have similar idiotypes. *Mol. Immunol.* **16:**1103.
7. Wikler, M., J.-D. Franssen, C. Collignon, O. Leo, B. Mariame, P. van de Walle, D. de Groote, and J. Urbain. 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.

8. Metzger, D. W., A. Miller, and E. E. Sercarz. 1980. Sharing of an idiotypic marker by monoclonal antibodies specific for distinct regions of hen lysozyme. *Nature (Lond.).* **287:**540.
9. Ju, S-T., B. Benacerraf, and M. E. Dorf. 1980. Genetic control of a shared idiotype among antibodies directed to distinct specificities. *J. Exp. Med.* **152:**170.
10. Kohno, Y., I. Berkower, G. Buckenmeyer, J. Minna, and J. A. Berzofsky. 1981. Shared idiotopes among monoclonal antibodies to distinct determinants of sperm whale myoglobin. *J. Supramol. Struct. Cell. Biochem. Suppl.* **5:**37.
11. Wysocki, L. J., and V. L. Sato. 1981. The anti-p-azophenylarsonate cross-reactive idiotypic family of strain A mice includes members with no reactivity toward *p*-azophenylarsonate. *J. Supramol. Struct. Cell. Biochem. Suppl.* **5:**38.
12. Dzierzak, E. A., E. A. Lerner, and C. A. Janeway, Jr. 1981. Regulation of Id-460: a predominant idiotype in the anti-DNP response. *J. Supramolec. Struct. Cell. Biochem. Suppl.* **5:** 40.
13. Bach, M., M. Wittner, D. Levitt, and H. Kohler. 1981. Demonstration of rare (unique) and frequent (shared) idiotopes in mice and humans using hybridoma anti-TEPC-15 antibodies. *Fed. Proc.* **40:**1007.
14. Woodland, R., and H. Cantor. 1978. Idiotype-specific T helper cells are required to induce idiotype-positive B memory cells to secrete antibody. *Eur. J. Immunol.* **8:**600.
15. Adorini, L., M. Harvey, and E. E. Sercarz. 1979. The fine specificity of regulatory T cells. IV. Idiotypic complementarity and antigen-bridging interactions in the anti-lysozyme response. *Eur. J. Immunol.* **9:**706.
16. Owen, F. L., S-T. Ju, and A. Nisonoff. 1977. Binding of idiotypic determinants of large proportions of thymus-derived lymphocytes in idiotypically suppressed mice. *Proc. Natl. Acad. Sci. U. S. A.* **74:**2084.
17. Bona, C., W. E. Paul. 1979. Cellular basis of regulation of expression of idiotype. I. T-suppressor cells specific for MOPC-460 idiotype regulate the expression of cells secreting anti-TNP antibodies bearing 460 idiotype. *J. Exp. Med.* **149:**592.
18. Jerne, N. K. 1974. Towards a network theory of the immune system. *Ann. Immunol. (Paris)* **125:**373.
19. Makela, O., and K. Karjalainen. 1977. Inherited immunoglobulin idiotypes of the mouse. *Immunol. Rev.* **34:**119.
20. Kelsoe, G., and J. Cerny. 1979. Reciprocal expansions of idiotypic and anti-idiotypic clones following antigen stimulation. *Nature (Lond.).* **279:**333.
21. Conger, J. D., G. K. Lewis, and J. W. Goodman. 1981. Idiotype profile of an immune response. I. Contrasts in idiotypic dominance between primary and secondary responses and between IgM and IgG plaque-forming cells. *J. Exp. Med.* **153:**1173.
22. Harvey, M. A., L. Adorini, A. Miller, and E. E. Sercarz. 1979. Lysozyme-induced T-suppressor cells and antibodies have a predominant idiotype. *Nature (Lond.).* **281:**594.
23. Benjamin, C. D., A. Miller, E. E. Sercarz, and M. A. Harvey. 1980. A predominant idiotype on anti-hen eggwhite lysozyme antibodies from diverse mouse strains. *J. Immunol.* **125:**1017.
24. Kohler, G., and C. Milstein. 1975. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature (Lond.).* **256:**495.
25. Trowbridge, I. S. 1978. Interspecies spleen-myeloma hybrid producing monoclonal antibodies against mouse lymphocyte surface glycoprotein, T200. *J. Exp. Med.* **148:**313.
26. Schulman, M., C. D. Wilde, and G. Kohler. 1978. A better cell line for making hybridomas secreting specific antibodies. *Nature (Lond.).* **276:**269.
27. Gefter, M. L., D. H. Margulies, and M. D. Scharff. 1977. A simple method for polyethylene glycol-promoted hybridization of mouse myeloma cells. *Somat. Cell Genet.* **3:**231.
28. Kipp, D., and A. Miller. 1980. Preparation of protein-conjugated red blood cells with

EDCI (modification). *In* Selected Methods in Cellular Immunology. B. B. Mishell and S. M. Shiigi, editors. W. H. Freeman and Co., San Francisco. 103.

29. Tung, A. S., S-T. Ju, S. Sato, and A. Nisonoff. 1976. Production of large amounts of antibodies in individual mice. *J. Immunol.* **116**:676.
30. Awdeh, Z. L., A. R. Williamson, and B. A. Askonas. 1968. Isoelectric focusing in polyacrylamide gel and its application to immunoglobulin. *Nature (Lond.).* **219**:66.
31. Cecka, J. M., J. A. Stratton, A. Miller, and E. E. Sercarz. 1976. Structural aspects of immune recognition of lysozymes. III. T cell specificity restriction and its consequences for antibody specificity. *Eur. J. Immunol* **6**:639.
32. Cunningham, A. J., and A. Szenberg. 1968. Further improvements in the plaque technique for detecting single antibody-forming cells. *Immunology.* **14**:599.
33. Metzger, D. W., A. Miller, and E. E. Sercarz. 1980. Idiotypic characterization of anti-lysozyme hybridomas. *Fed. Proc.* **39**:572.
34. Herzenberg, L. A., K. Okumura, H. Cantor, V. L. Sato, F. Shen, E. A. Boyse, and L. A. Herzenberg. 1976. T-cell regulation of antibody response: demonstration of allotype-specific helper T cells and their specific removal by suppressor T cells. *J. Exp. Med.* **144**:330.
35. Janeway, C. A., R. A. Murgita, F. I. Weinbaum, R. Asofsky, and H. Wigzell. 1977. Evidence for an immunoglobulin-dependent antigen-specific helper T cell. *Proc. Natl. Acad. Sci. U. S. A.* **74**:4582.
36. Sakato, N., H. Fujio, and T. Amano. 1980. Idiotypic analysis of antibodies to hen egg-white lysozyme (HEL). I. Occurrence of species-specific cross-reactive idiotypes of antibodies directed to distinct regions of HEL in guinea pigs. *J. Immunol.* **124**:1866.
37. Semma, M., N. Sakato, H. Fujio, and T. Amano. 1981. Idiotypic analysis of antibodies to hen egg-white lysozyme (HEL). II. Distribution and frequency of occurrence of idiotypes shared by A/J anti-HEL antibody. *Immunology.* **42**:45–51.
38. Maizels, R. M., J. A. Clarke, M. A. Harvey, A. Miller, and E. E. Sercarz. 1980. Epitope specificity of the T cell proliferative response to lysozyme: T proliferative cells react predominantly to different determinants from those recognized by B cells. *Eur. J. Immunol.* **10**:509.
39. Sercarz, E. E., and D. W. Metzger. 1980. Epitope-specific and idiotype-specific cellular interactions in a model protein antigen system. *Springer Semin. Immunopathol.* **3**:145.