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Immunology

Enhancing antibody: A novel component of the immune response

(immune complexes/networks/idiotype/anti-idiotype/rheumatoid factor)

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ABSTRACT **Current descriptions of the immune response identify two classes of antigenic stimuli that result in the production of specific antibody: (*i*) exogenous antigens and (*ii*) endogenous variable-region determinants of the immune system. We expand this scheme to include a third class of antigenic stimulus—new determinants created by the binding of antibody to antigen. This paper describes a set of monoclonal antibodies which arose after repeated immunization with antigen alone but which bound antibody–antigen complexes. These antibodies recognize determinants on the antibody portion of the complexes that were expressed as a consequence of antigen binding. Antibodies of this general type, "enhancing antibodies," which can strengthen antibody–antigen and idiotypic–anti-idiotypic antibody interactions, may play important regulatory and effector roles in the immune response. We suggest a model that predicts the occurrence and specificity of different classes of such antibodies and provides a conceptual framework that gives a straightforward explanation of the appearance in the immune response of rheumatoid antibodies and of antibodies that bind cooperatively to antigen.**

What roles do antibodies play in the regulation of the immune response? The network theory of the immune system (1–7) predicts that, in response to an antigen, not only are idiotypic antibodies synthesized but also anti-idiotypic antibodies, recognizing the new variable (V) regions as foreign determinants. In this report we identify a further class of responding antibodies that recognizes some aspect of the interaction between idiotypic and anti-idiotypic antibodies (idiotype–anti-idiotype interactions). These third party elements we call "enhancing" antibodies because they can be recognized through their ability to strengthen not only idiotype–anti-idiotype interactions but also antigen—antibody interactions in general. These antibodies may be directed to new determinants revealed on the antibody molecules as they interact and change their conformation or they may be directed to the new tertiary structure created by the binding of V regions to one another. In either case, enhancing antibodies can serve as an additional "glue" to increase the sensitivity of the interaction of V regions on B and T cells.

Our identification of enhancing antibodies arose during a study of the arsonate response in the A/J strain mouse. In response to the hapten *p*-azophenylarsonate (Ars), this strain produces antibodies that express a major crossreactive idiotype (CRI) (8). This unusual immune response provides a means of analyzing the role of idiotype—anti-idiotype interactions in the regulation of antibody synthesis, which is facilitated by the availability of monoclonal antibodies that express CRI (9, 10) and anti-CRI specificities (refs. 9 and 10; P. Hornbeck, personal communication).

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MATERIALS AND METHODS

Animals. BALB/c CRL mice and CD rats were purchased from Charles River Laboratories. A/J mice were purchased from Jackson Laboratory. C.AL-20 mice were the gift of M. Weigert. All animals were maintained in the Animal Facilities of The Biological Laboratories, Harvard University.

Antigens and Iodinations. Ars-conjugated ovalbumin (Ars-Ova) was prepared by the method of Nisonoff (11). Iodinations were carried out by the method of Hunter and Greenwood (12).

Antisera. Mouse anti-rat Ig ascites were raised in BALB/c mice as described (13). Normal rat Ig was purified from serum by ammonium sulfate precipitation and DEAE ion-exchange chromatography (14). Antibodies specific for normal rat Ig were prepared by absorption onto normal rat Ig-coupled Sepharose 4B (15), followed by elution with 3 M KSCN. Preparation of affinity-purified A/J anti-Ars antibodies has been described (10).

Monoclonal CRI Antibodies. Ars-nonbinding CRI antibodies 3A4 (γ_1,κ) and 1F6 (γ_1,κ), produced from an A/J mouse immunized with hybridoma 5Ci (10), were provided by Lawrence Wysocki. Both were highly purified for amino acid sequence determination. Affinity-purified monoclonal CRI antibodies 16–46 (γ_{2a},κ), 31–62 (γ_{2a},κ), and 36–65 (γ_1,κ) were gifts of Ann Marshak-Rothstein.

Monoclonal Anti-CRI Antibodies. 5Ci is a hybridoma resulting from the fusion of NS-1 mouse myeloma cells and spleen cells from a CD rat immunized with CRI antibodies (10). 5Ci protein was purified from ascites fluid by ammonium sulfate precipitation and DEAE chromatography. It was ≈80% pure as judged by $NaDodSO_4$/polyacrylamide gel electrophoretic analysis. Monoclonal BALB anti-CRI (16) antibody was the kind gift of Ann Marshak-Rothstein. It was affinity purified on protein A-Sepharose. Both 5Ci and BALB anti-CRI antibodies bind to all of the monoclonal CRI antibodies listed above and to A/J anti-Ars antibodies found in immune serum. AD8 monoclonal anti-CRI antibody, a gift of Peter Hornbeck and George Lewis (University of California, San Francisco), was produced from the fusion of CRI antibody-immune Lewis rat spleen cells and the Y3-Ag1,2,3 rat tumor cell line (17). It also binds to all the CRI antibodies used in this study.

Hybridoma Production. Monoclonal antibodies were prepared by a modification of the method of Gefter *et al.* (18). A single male CD rat was immunized with 250 μg of affinity-purified A/J anti-Ars antibody in complete Freund's adjuvant. Boosting doses of the antigen were administered in incomplete Freund's adjuvant 4, 8, and 12 wk after the initial injection. The

Abbreviations: Ars, azophenylarsonate; PVC, polyvinyl chloride; Ova, ovalbumin; V, variable; CRI, immunoglobulin molecules expressing the crossreactive idiotype.

rat was then rested for 6 mo before a final booster of 100 μg of A/J anti-Ars in phosphate-buffered saline 3 days prior to fusion. Fusion was performed with 1×10^8 spleen cells and 2×10^7 Y3-Ag1,2,3 cells (17). After fusion, the cells were immediately distributed into nine 96-well microtiter trays (No. 3596, Costar, Cambridge, MA). After 10 days, visible hybridoma colonies were present in 20% of the wells. One resulting enhancing-antibody clone (22C9) was recloned and grown as an ascites in pristane-primed BALB/c mice that had been x-irradiated (600 rad).

Radioimmunoassays. Three types of solid-phase radioimmunoassays (19, 20) were used. In all cases, polyvinyl chloride (PVC) plastic wells were coated with 50 μl of antibody or antigen in phosphate-buffered saline (≥5 μg/ml) for 1–5 hr. The solution was removed, and the wells were washed repeatedly with 0.5% human serum albumin to saturate the protein-binding sites of the plastic. All subsequent incubations were at room temperature in the presence of 0.5% human or bovine serum albumin in phosphate-buffered saline. The initial screen for rat anti-CRI-antibody and enhancing-antibody hybridomas was done as follows. Wells were coated with affinity-purified A/J anti-Ars antibodies and washed. A mixture (50 μl) containing 10 ng of ^{125}I-labeled 5Ci anti-CRI antibodies (35,000 cpm) and 25 μl of culture supernatant was added to each well and incubated for 2 hr. After this period, microtiter wells were emptied and washed, and bound radioactivity was measured on a Packard "Auto-gamma" gamma counter.

The second and third types of assay involved immobilizing rat enhancing antibodies onto PVC wells either directly (as described) or indirectly by incubating them in wells previously coated with mouse anti-rat Ig. In the latter case, the coated wells were blocked with a solution containing human serum albumin and then incubated with culture supernatants for 2–4 hr. After the wells were washed, they were incubated for 30 min with a solution made 2% in normal rat serum and 2% in normal C.AL-20 mouse serum in order to block the anti-Ig sites on the wells. All subsequent incubations in wells coated with mouse anti-rat Ig were also done in the presence of normal sera at the same concentrations. The effect of various proteins on the ability of PVC-immobilized enhancing antibodies to bind to ^{125}I-labeled CRI or anti-CRI antibodies was then assayed by adding to each well 50 μl of a mixture containing 120–200 ng of labeled antibody per ml plus different concentrations of the protein in question. After an 8-hr incubation period, wells were washed extensively and the amount of bound ^{125}I was determined.

Preparation of F(ab')$_2$ Fragments. F(ab')$_2$ fragments of 5Ci monoclonal anti-CRI antibody were prepared as described (21). These fragments were clearly bivalent because they could mediate the binding of ^{125}I-labeled 3A4 monoclonal CRI to 3A4-coated PVC wells (data not shown).

Table 1. Competition assay for anti-CRI antibody detects anti-CRI and enhancing antibodies

Hybridomas		^{125}I-Labeled 5Ci bound,* cpm	% inhibition
Negative controls	Medium	728	0
	24H2	702	3.6
Inhibition controls	5Ci (5 μg/ml)	111	84.7
	5Ci (1 μg/ml)	371	49.0
Inhibiting antibodies	28H12	435	32.0
	22C7	39	94.6
	27A3	360	50.5
Enhancing antibodies	22C9	1,614	−121.7
	24D3	981	−34.6
	24C5	2,355	−223.5
	25C11	1,997	−173.8

Monoclonal antibodies were tested for the ability to block the binding of ^{125}I-labeled anti-CRI antibody 5Ci in solution to affinity-purified A/J anti-Ars antibodies immobilized on PVC. Hybridoma supernatants were used at a final concentration of 50%. Each number is the mean of triplicate wells.

* ^{125}I-Labeled anti-CRI antibody bound to CRI antibody-coated wells.

RESULTS

Discovery of Enhancing Antibodies. In an attempt to obtain monoclonal anti-CRI antibodies, we fused spleen cells from a CRI immunized rat with the rat myeloma line Y3-Ag1,2,3 and assayed culture supernatants from hybridomas for the ability to inhibit the binding of a specific rat anti-CRI (5Ci) to affinity-purified anti-Ars A/J antibodies containing the CRI. Out of 166 hybridomas, three produced antibodies that inhibited the CRI–anti-CRI interactions, whereas, to our surprise, 16 hybridoma supernatants increased (30–300%) the amount of radioactive anti-CRI antibody bound. Four of these hybridomas were chosen for further study. Table 1 shows the results of the assays using supernatants from these four cell lines. Clearly a factor is present that fixes the labeled anti-CRI antibody to the PVC wells.

Table 2 shows that the enhancement is inhibited by Ars-Ova, which will block the binding of the anti-idiotypic antibody to the CRI antibody, but is not by normal mouse serum or by MOPC-21, a (γ_1,κ) antibody. Thus, the enhancing factor sees the anti-CRI–CRI complex; it does not see epitopes on the normal mouse antibodies. Because the rat 5Ci monoclonal antibody contains the MOPC-21 κ chain (derived from the NS-1 fusion parent), this experiment rules out the possibility that antigenic similarities between the V region light chains of the CRI and the ^{125}I-labeled 5Ci antibody were crosslinked by the enhancing factor.

Table 2. Inhibition of the effect of enhancing antibodies*

Inhibitor	Enhancing antibody in solution				
	Medium	22C9	24D3	24C5	25C11
—	2,318 (100)	4,806 (207)	3,031 (131)	5,820 (251)	4,911 (211)
MOPC-21	2,224 (96)	4,424 (191)	2,474 (107)	5,578 (241)	4,747 (205)
5Ci	562 (24)	729 (31)	543 (23)	698 (30)	683 (29)
N BALB/c serum	2,110 (91)	4,115 (178)	2,748 (119)	5,218 (225)	4,492 (194)
N C.AL-20 serum	2,078 (90)	4,429 (191)	2,697 (116)	5,478 (236)	4,461 (192)
Ova-Ars	429 (19)	1,491 (64)	741 (32)	1,935 (83)	1,821 (79)
Ova	2,159 (93)	5,127 (221)	2,987 (129)	5,978 (258)	4,728 (204)

* Values are cpm of ^{125}I-labeled anti-CRI antibody 5Ci bound to anti-Ars-coated PVC wells; values in parentheses express the percentage of medium control. Assay scheme is the same as in Table 1, except that various inhibitors of enhancement were added. The final concentrations of inhibitors were: MOPC-21 (10 μg/ml), 5Ci (1 μg/ml), Ova-Ars and Ova (200 μg/ml), and normal (N) BALB/c and C.AL-20 sera (1%). The final concentrations of enhancing antibody supernatants or medium in solution was 50%.

Are these enhancing factors antibodies? We shall show that the enhancing factors can be bound by anti-rat Ig. Furthermore, when the supernatants of [^{35}S]methionine-labeled cells were examined by NaDodSO$_4$/polyacrylamide gel electrophoresis (data not shown), only two labeled bands appeared, migrating as would a μ heavy chain and a light chain. We infer that these hybridomas are secreting an IgM that interacts with the idiotype–anti-idiotype complex; we shall call such antibodies (of any class) "enhancing antibodies." To confirm further that the enhancing antibody interacts with a complex and not with the two components singly, we performed an Ouchterlony experiment. Fig. 1 shows the precipitation pattern between three wells containing rat monoclonal anti-CRI 5Ci, mouse monoclonal CRI 3A4, and one of the four enhancing antibodies, 22C9. No line of precipitation appears between each of the separate wells and 22C9 (although because 22C9 is IgM, it should precipitate); however, a displaced line of precipitation appears where all three components meet.

Immobilized Enhancing Antibodies Recognize Idiotypic–Anti-idiotypic Antibody Complexes. A clearer assay, with a greater range over background, is to immobilize the enhancing antibodies on the PVC wells, either directly or by binding to mouse anti-rat Ig, and to expose them to complexes in which either the idiotypic or the anti-idiotypic antibody is labeled. Table 3 shows an experiment in which labeled idiotypic antibody (mouse monoclonal CRI 3A4) is exposed to the enhancing antibody alone or is complexed to 5Ci (a rat anti-CRI), to 22C7 or 28H12 (both rat anti-CRI monoclonal antibodies that arose in the fusion that generated the enhancing antibodies), or to a monoclonal BALB anti-CRI antibody. In the presence of the various anti-idiotypic antibodies, 5- to 35-fold increases in binding appeared. This experiment shows that (*i*) different anti-idiotypes, including autologous ones, are recognized; (*ii*) the enhancing antibodies differ in detail in their interaction with the different complexes, and (*iii*) a single animal can produce anti-CRI and enhancing antibodies.

We examined in detail the binding properties of one enhancing antibody, 22C9. Labeled 5Ci (the rat anti-CRI antibody) alone was not bound by 22C9, but, as greater and greater

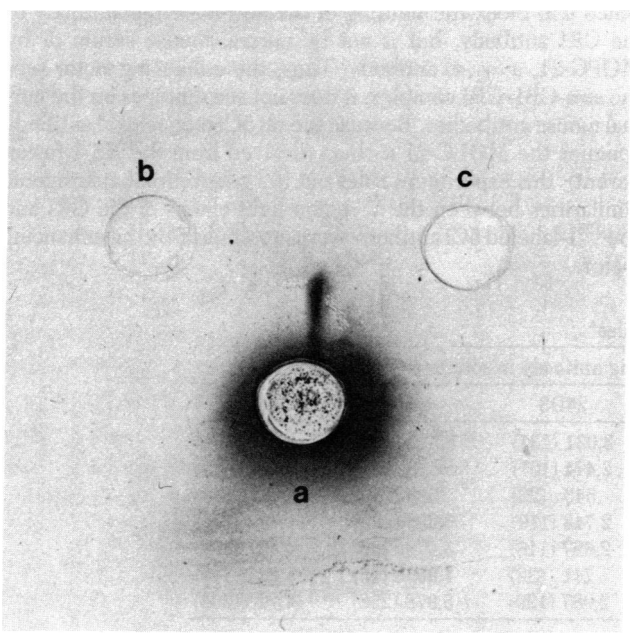

FIG. 1. Coprecipitation among enhancing antibody 22C9 (well a), 5Ci anti-CRI antibody (well b), and CRI IgG1 3A4 antibody (well c). Each well received 10 μl of a solution (1 mg/ml) of purified protein. Note that no precipitin bands appeared between any two wells.

Table 3. Enhancer antibodies bind autologous antibody–antigen complexes

Anti-CRI antibody in solution	^{125}I-Labeled CRI antibody 3A4 bound,* cpm			
	Enhancing antibody on solid phase			
	22C9	24D3	24C5	25C11
Medium	332	207	197	745
22C7 (50% sup)	1,705	98	2,671	12,497
28H12 (50% sup)	3,627	2,387	4,152	6,313
5Ci (1 μg/ml)	5,109	575	7,658	17,569
BALB/c (1 μg/ml)	1,438	3,343	477	9,133

* Enhancing antibodies from culture supernatants (sup) were immobilized on PVC dishes coated with mouse anti-rat Ig. Their ability to bind to ^{125}I-labeled CRI 3A4 antibody in solution in the presence of autologous (22C7, 28H12) or heterologous (5Ci, BALB αCRI) monoclonal anti-CRI antibodies was tested. This assay was done in the presence of 2% normal rat serum and 2% normal mouse serum to prevent the binding of Ig in solution to the anti-rat Ig. CRI bound to control wells with immobilized 22C7, 28H12, or medium was 545, 1,254, and 112 cpm, respectively.

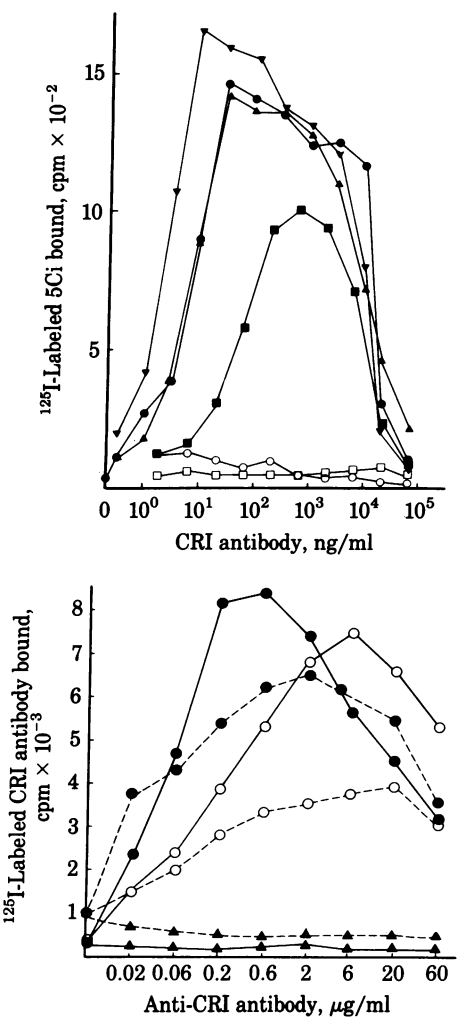

FIG. 2. Isotype specificity of 22C9 enhancer antibodies. In this experiment, PVC dishes were coated directly with diluted 22C9 ascitic fluid. (*A*) The ability of 22C9 to bind to ^{125}I-labeled 5Ci anti-CRI antibody alone or in the presence of different concentrations of a number of CRI antibody preparations was determined. The final concentration of ^{125}I-labeled 5Ci was 120 ng/ml. Added CRI preparations: (▼), 3665 (γ_1,κ); (▲), 1F6 (γ_1,κ); (●) 3A4 (γ_1,κ); (□), 1646 (γ_{2a},κ); (○), 3162 (γ_{2a},κ); and (■), affinity-purified A/J anti-Ars. (*B*) The binding by 22C9 of ^{125}I-labeled 3665 CRI antibody (---) or ^{125}I-labeled 3A4 (——) (both at 200 ng/ml) in the presence or absence of anti-CRI proteins. Added monoclonal anti-CRI antibodies: (●), 5Ci; (○), BALB; (▲), AD8.

amounts of three different mouse IgG1 CRI monoclonal antibodies were added, a striking maximum of binding occurred near the equimolar point (120 ng/ml) for the formation of idiotype—anti-idiotype complexes (Fig. 2). Affinity-purified A/J anti-Ars antibodies also facilitated the binding of 5Ci under these conditions.

Fig. 2 shows that 22C9 can bind complexes made by CRI IgG1 and anti-CRI IgG1 but not complexes in which either the CRI or anti-CRI antibody is of another IgG subclass. Two mouse CRI antibodies of the IgG2a subclass did not form complexes recognized by 22C9, although they did form stable complexes with 5Ci (ref. 10; unpublished results). Similarly, 22C9 bound complexes formed between antibodies of two different mouse IgG1 idiotypes and either 5Ci or a monoclonal BALB anti-CRI (γ_1, κ) antibody but not complexes between antibodies of those same mouse idiotypes and AD8, an anti-CRI antibody derived from a Lewis rat (Fig. 2*B*). [AD8 and 5Ci have comparable affinities for the CRI-bearing proteins used in this study (unpublished results).] Because the idiotypic antibody may well be in a similar conformation when bound to either of the rat anti-idiotypic antibodies, this last observation argues that the enhancing antibody sees (*i*) a joint site created by the interacting V regions of the idiotypic and the anti-idiotypic antibody, (*ii*) a site created by a conformational change in the anti-idiotypic antibody, or (*iii*) sites created by conformational changes in both the idiotypic and the anti-idiotypic antibodies so that both sites have to be present for the binding to be strong enough to be detected. We attacked each of these possibilities in turn.

If these enhancing antibodies bound to sites created by the complexing of the idiotypic–anti-idiotypic V regions, then the Fab fragment of 5Ci should be able to replace 5Ci in inducing binding of labeled idiotypic antibody by the enhancing antibody. However, anti-CRI(Fab)–CRI complexes do not bind the enhancing antibody (data not shown). Because one might worry that the affinity of the Fab fragment is too low, we isolated $F(ab')_2$ fragments of 5Ci. Fig. 3 shows that such $F(ab')_2$ fragments do not replace 5Ci IgG as the anti-idiotypic portion of the complex. This last (consistent with the fact that the IgG2a CRI molecules do not work) rules out the possibility that these enhancing antibodies are seeing the complexed V regions. Therefore, 22C9 must recognize a new determinant created by a conformational change in the complexing antibodies. The experiment of Fig. 3 shows that a change in the Fc portion of 5Ci, the anti-idiotypic antibody, must be involved. Because a mouse anti-idiotypic antibody of the IgG1 class can substitute for 5Ci (Fig. 2*B*), the new determinant revealed by a conformational change must also be on the Fc piece of mouse IgG1 antibodies. Complexes in which only the Fc portion of the mouse CRI IgG1 is intact cannot be bound by 22C9 (Fig. 3). Thus, the Fc portions of both the idiotypic and the anti-idiotypic antibodies are necessary for recognition by 22C9. [We were unable to test this conclusion directly because the CRI protein precipitates under conditions required for preparation of $F(ab')_2$ fragments.]

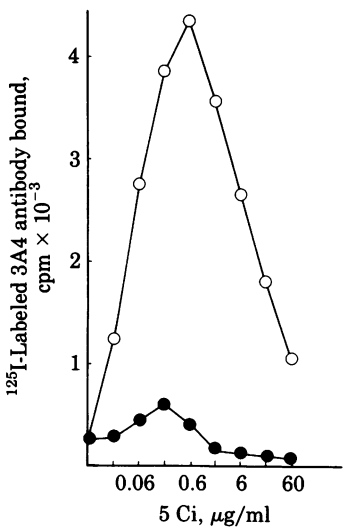

FIG. 3. The binding of 22C9 enhancing antibody to 3A4 CRI–5Ci anti-CRI antibody complexes requires intact 5Ci. Diluted 22C9 ascites fluid was directly bound to PVC dishes and assayed for the ability to bind to ^{125}I-labeled 3A4 in the presence of various concentrations of 5Ci IgG (○), or 5Ci $F(ab')_2$ (●).

We conclude that 22C9 antibodies most likely interact with a site on a γ_1 Fc fragment that is revealed when that antibody interacts with an antigen. In addition, more than one such site must be present in the complex. This interpretation would explain why neither complexes of CRI γ_1 with AD8 nor complexes of CRI γ_{2a} with 5Ci bind to 22C9 (Fig. 2); presumably the anti-idiotypic antibody AD8 is not of the γ_1 subclass.

DISCUSSION

In this report we describe a set of antibodies that have binding specificity for autologous antibody bound to antigen—enhancing antibodies. These antibodies arise as a natural response to a new antigenic determinant present in the antibody–antigen complex and recognized as "foreign" by the immune system.

In a fusion to produce hybridomas from spleen cells of a rat immunized with mouse idiotypic antibody, 3–4 times as many hybridomas were produced that bound to idiotype–anti-idiotype complexes than hybridomas that bound to idiotypic antibody alone. Four such enhancing antibodies were shown to bind autologous, isologous, and heterologous anti-idiotype–idiotype complexes and to be of the IgM isotype. One of these antibodies, 22C9, was shown to bind complexes of anti-idiotypic IgG1 and idiotypic IgG1. This antibody had no specificity for immune complexes formed between anti-idiotypic (Fab) fragments and idiotypic antibody or between anti-idiotypic $F(ab')_2$ fragments and idiotypic antibody; specificity was for anti-idiotype–idiotype complexes formed between intact IgG1 molecules. These data strongly suggest that enhancing antibody 22C9 recognizes a determinant on the Fc fragment of IgG1 molecules that is induced or exposed as a consequence of antigen binding.

The monoclonal enhancing antibodies described herein are strikingly reminiscent of classical rheumatoid factors (22). Rheumatoid factors in humans are autoantibodies, usually IgM, which recognize antigenic determinants on the Fc portion of IgG. Generally such rheumatoid antibodies bind more tightly to aggregated or partially denatured IgG than to IgG in the native, deaggregated form (23, 24). Some workers have suggested that rheumatoid factors preferentially recognize IgG bound to antigen (23, 25–27). Though often associated with a variety of pathological conditions, rheumatoid factors can be detected in high titers in a small percentage of healthy persons, and they are frequently found in the sera of patients shortly after the occurrence of acute bacterial and viral infections or, indeed, whenever antibody–antigen complexes may be present in the circulation (27–29).

We suggest that rheumatoid factors in general do not represent the products of "forbidden clones" of B cells that are autoreactive to self Ig but rather arise from the stimulation of cells with binding specificity to new antigenic determinants created or exposed by the formation of antibody–antigen complexes.

Precursors of this type of enhancing antibody–producing cells may comprise a very large subpopulation of B cells. Metzger (30) observed that a large percentage of IgM proteins isolated from patients with Waldenstrom's macroglobulinemia have

rheumatoid factor activity. In addition, Dresser (31) has described experiments that show that over 75% of lipopolysaccharide-stimulable IgM antibody-forming cells in mice produce rheumatoid factor. Thus, in any individual, a large percentage of B cells may be specific for altered self Ig—i.e., Ig which is bound to antigen. Just as helper and certain effector T cells recognize antigen in the context of self components of the major histocompatibility complex, one aspect of immune regulation, so also many B cells may have an IgH-linked specificity for antibody–antigen complexes and so play a regulatory role.

Although the enhancing antibodies described in this study were induced by idiotype–anti-idiotype complexes, we recently have isolated enhancing antibodies from a mouse immunized with a nonimmunoglobulin antigen Ars-*Limulus polyphemus* hemocyanin (Ars–LPH). Preliminary examination of one such antibody shows it to be an IgG3 that can bind complexes of Ars–LPH and idiotypic antibody. Clearly this enhancing antibody has a different specificity from that of 22C9.

It is reasonable that antibody–antigen complexes can act as antigens in their own right in the elicitation of antibodies. These complexes are taken up by macrophages and contain "carrier" determinants in the form of exogenous antigens. As one expects, and as this study shows, antigen–antibody complexes have antigenic determinants that are not present on antigen or antibody alone. Theoretically, three possible types of antibody might be produced in response to antibody–antigen complexes: (*i*) those that recognize a change in conformation of the antibody moiety of the complex induced by antigen binding (in this category fall the antibodies described in this paper and also the rheumatoid-factor antibodies); (*ii*) those that recognize a conformational change in the antigen induced by another antibody binding to the antigen (refs. 32 and 33); such antibodies often have substantial affinity for antigen alone, but bind better along with a second antibody specific for a different part of the same antigen; and (*iii*) those specific for neoantigens created by the close juxtaposition of antibody and antigen (or two V regions). In this case, the recognized site would be composed of both the foreign antigen and an idiotypic antibody.

Of what physiological utility might enhancing antibodies be? Several possibilities arise.

(*i*) Enhancing antibodies bound to antigen–antibody complexes may improve the ability of the complex to fix complement and, therefore, to trigger cytolysis in the case of cellular antigens or clearance through opsonization in the case of soluble antigens. Thus, an enhancing antibody of the IgM isotype could amplify the cytotoxicity of IgG bound to an antigen.

(*ii*) Enhancing antibodies stabilize antigen–antibody complexes, thus increasing the apparent affinity of antibody for antigen. Therefore, the presence of circulating enhancing antibodies would result in improved clearance of antigen from the circulation. This also suggests that enhancing antibodies may contribute in part to the observed avidity maturation of antisera specific for antigen in individuals that are hyperimmunized to antigen over an extended period of time.

(*iii*) Enhancing antibodies may increase the antigen-recognizing sensitivity of the immune system. This could be accomplished by the enhancing antibody stabilizing antibody–antigen complexes in which the antibody component is the surface Ig of B cells. This would have the effect of increasing the apparent affinity of the B cell for the antigen and, thus, provide B cells with appropriate Ig determinants a competitive advantage in antigen binding over other antigen-specific B cells that do not have determinants recognizable by enhancing antibodies. Furthermore, we would not be surprised if there turn out to be enhancing antibodies that recognize new determinants in such a way as to enhance specific B cell–T cell collaborations, or helper–suppressor cell interactions.

Our hypothesis identifies an additional player in the immune system that is sensitive to the presence of the conjunction of interacting molecules or V regions and, thus, serves to increase the sensitivity and specificity of molecular and cellular interactions.

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