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UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN
College of Veterinary Medicine

MASTER

Contract No. AT(11-1)-1628
Final Report

Title of Project:

Immunologic Recognition and Immunologic Memory.

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Technical Report:

The objective of the project was to test the proposition that circulating antibodies perform a regulatory function in antibody formation. The working hypothesis was that the ratio of the concentration of antigen to that of circulating antibody is the controlling factor which determines whether antibody production will or will not result from exposure to the antigen, as well as the magnitude of the antibody response. Thus, preformed, circulating antibodies would constitute the immunologic recognition system and would be responsible for immunologic memory effects. This hypothesis was enunciated by Eisen and Karush (1) and supported by work done in this laboratory (2-8) prior to the initiation of this project.

Work conducted under the project attempted to assess the role of antibody concentration in the immune response and in immunologic tolerance. This was accomplished by taking advantage of experimental situations in which the concentration of antibody, natural or immune, was altered, and by measuring the immune response and the influence exerted upon it by administration of exogenous antibody or immunoglobulin. In addition, new techniques that were devised to meet certain experimental requirements proved valuable for the investigation of a number of basic immunologic problems.

a. Termination of immunologic tolerance by antigen-antibody complexes.

According to the model of Eisen and Karush (1) immunologic tolerance is induced when the concentration of circulating antigen is much greater than that of the corresponding natural antibody. This would result in the formation of trimolecular complexes (Ag_2 Ab), made of one molecule of antibody combined with two molecules of antigen. Such complexes would not gain access to the immunologically competent cells, but would be rapidly degraded and eliminated. A specific prediction of the model is that tolerant animals should contain cells immunologically competent to respond to the tolerated antigen. This was tested by exposing in vitro spleen cells from tolerant mice to either the tolerated antigen alone or to the tolerated antigen mixed with specific antibody.

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The cells were then injected back to the mice from which they had been derived. Ninety percent of the animals whose spleen cells had been exposed to antigen-antibody complexes lost their tolerance, whereas 55% of the mice whose spleen cells had been exposed to antigen alone remained tolerant (9).

b. Passive transfer of the action of Freund's adjuvant.

The antibody response to soluble antigens is greatly enhanced when the antigen is emulsified with complete Freund's adjuvant. Humphrey (10) has shown that rabbits injected with Freund's adjuvant alone have greatly increased levels of serum globulin. Furthermore, the antibody response of rabbits to fluid diphtheria toxoid emulsified with complete Freund's adjuvant has many of the characteristics of the secondary response (7). On the basis of these findings, and in line with the model of Eisen and Karush (1), it was hypothesized that Freund's adjuvant may increase the concentration of natural antibodies non-specifically, thus causing more antibodies to be available for the formation of the immunogenic Ag Ab complexes and increasing the effectiveness of the antigen. If this were so, it should be possible to passively transfer the activity of Freund's adjuvant with the serum of adjuvant-treated animals. This was indeed accomplished (7). Moreover, the effectiveness of the serum from adjuvant-treated donor rabbits in enhancing the antibody response of the recipients was correlated with the increase in globulin concentration of the sera (7). Further work (11) demonstrated that purified immunoglobulin G (IgG) from the serum of adjuvant-treated rabbits was as effective as whole serum in enhancing the antibody response of recipient rabbits, and that administration of Freund's adjuvant caused an increase in the concentration of IgG, but not of other immunoglobulins.

c. Induction of immunologic paralysis and immunity in newborn offspring of immunologically paralyzed mice.

If immunologic tolerance results from the specific removal by excess antigen of the corresponding natural antibodies, offspring of tolerant mothers should fail to acquire maternal antibodies of that specificity. Thus, the concentration of natural antibody specific for the tolerated antigen should be lower in the newborn offspring of tolerant mice than in offspring of normal mice. As a consequence, less antigen should be required to establish the antigen excess necessary for induction of tolerance in mice newly born of tolerant mothers than in normal newborn mice. A similar relentionship should obtain in the two types of mice as far as the immunogenic doses of antigen are concerned.

To test this hypothesis, newborn offspring of normal mice and of mice immunologically paralyzed with pneumococcal polysaccharide type 3 (S3) were injected with a wide range of doses of the polysaccharide. One week later all mice were challenged with 100 minimal lethal doses of Diplococcus pneumoniae type 3 to assess their immune status. It was found that the immunizing and paralyzing doses of polysaccharide were tenfold lower in offspring of paralyzed mice than they were in normal mice (12). Furthermore, it was found that the difference in susceptibility to the induction of paralysis and immunity between offspring of paralyzed and of normal mice decreased gradually with age (13). Administration of either specifically purified anti-S3 antibody or of normal IgG restored the offspring of paralyzed mice to the same level of susceptibility to the induction of both paralysis and immunity as that exhibited by normal mice (13). The biologic activity of normal IgG was removed by a specific

immunoadsorbent, thereby confirming that the effect of normal IgG was due to specific anti-S3 antibody (13). Similar, but more quantitative, results were obtained when paralysis and immunity to S3 were measured by hemagglutination (13) or by enumeration of antibody-forming cells with a localized hemolysis-in-gel technique (14), rather than by susceptibility or resistence to challenge by pneumococcus. It was concluded that a relative deficiency of natural anti-S3 antibodies was indeed responsible for the altered immunologic behavior of offspring of paralyzed mice, and that the results obtained are compatible with, and explained by, a role of preformed antibody in the regulation of antibody formation.

d. The use of the localized hemolysis-in-gel procedure in immunologic investigations.

Jerne and Nordin (15) demonstrated that lymphoid cells from a donor immunized with heterologous erythrocytes produce localized areas of hemolysis upon incubation in agar gel containing the immunizing erythrocytes and complement. This technique permits the detection and enumeration of lymphoid cells synthesizing specific antibodies, and has been very useful for the study of the immune response. The method, as originally described, is limited to erythrocytic antigens and cells producing antibodies against them.

The antibody plaque procedure was adapted to the detection and enumeration of cells producing antibodies against foreign IgG (16), protein antigens such as ovalbumin (17) and allotypic determinants of immunoglobulins. It was found that sheep erythrocytes sensitized with the IgG fraction of commercial rabbit anti-sheep hemolysin were not hemolyzed directly by complement, but were hemolyzed after treatment with antirabbit globulin and complement. Erythrocytes so sensitized were plated in agar containing spleen cells from mice immunized with rabbit IgG. After incubation and addition of complement, hemolytic plaques developed around spleen cells producing antibodies specific for rabbit IgG (16). When other antigens, such as ovalbumin (17), ribonuclease, lysozyme, bovine serum albumin or the hapten dinitrophenol (19), were linked covalently to the IgG hemolysin of rabbit origin, hemolytic plaques were obtained with cells producing antibodies against these antigens or haptens. The use of IgG hemolysin from rabbits of defined allotypes allowed the development of plaques by rabbit cells producing anti-allotype antibodies (18). A useful general feature of this procedure is the ability of the specific soluble antigen to inhibit plaque formation when it is incorporated into the agar. This general procedure has been or is being used to investigate the relationship between heavy chain allotypic determinants of rabbit IgG and IgM (18), the phenomenon of allotype suppression in rabbits (19), the production of antibodies of two different specificities by single rabbit cells (19), the cross reactivity among avian lysozymes from different species (19), the localization of antigenic determinants of bovine pancreatic ribonuclease (19), the relationship of length of immunization to changes in association constant and heterogeneity of antibody (19), and the mechanism of immunosuppression by a serum alpha globulin (19). A sensitive, quantitative, hemolysis-inhibition assay, capable of detecting less than one nanogram of chicken lysozyme, and comparable amounts of other antigens, has also been developed (19).

e. References:

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