Contract No. AT(11-1)-1628

Technical Progress Report

RELEASED FOR ANNOUNCEMENT
IN NUCLEAR SCIENCE ABSTRACTS

For the Period May 15, 1966, through February 1, 1967

MAR 9 1967

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1. Title of Project:

Immunologic Recognition and Immunologic Memory

2. Institution:

College of Veterinary Medicine, University of Illinois

Urbana, Illinois 61801

3. Progress Report:

a. Termination of the tolerant state by administration of antigen-antibody complexes. A reprint of an article entitled "Termination of Immunologic Tolerance in Mice by Antigen-Antibody Complexes" by F. E. Hemphill, D. Segre and W. L. Myers, Proc. Soc. Exp. Biol. Med., 123:265, 1966, is attached and will serve as a report of progress in this phase of the project.

b. Role of thymus in immunologic maturation. Thymectomy of mice at birth has been shown to adversely affect various immunologic responses and to result in wasting disease (1,2). If mice thymectomized at birth are grafted with an intact thymus from a neonatal donor (1) or injected with sufficient numbers of adult thymus cells (3), they are protected from the expected immunologic effects. It has been reported that thymectemized mice which received thymus tissue enclosed in cell-impermeable Millipore chambers were protected to a large degree from immunologic impairment (4). This finding suggests that at least part of the thymic function is mediated by a humoral factor.

The hypothesis that the thymic humoral factor may be natural antibody globulin was formulated on the basis of results obtained in this laboratory (5). To test this hypothesis, two groups of C57BL/6J mice were

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thymectomized at birth. Mice of one group received weekly intraperitoneal injections of 100 μ g of immuno-globulin G (IgG) derived from either rabbit thymuses or rabbit serum. Thymectomized mice of the other group, as well as sham-thymectomized mice of a third group, received no treatment. Individual mouse weights were measured weekly. All mice received injections of bovine serum albumin (BSA) and ovine red blood cells (ORBC) at 5 and 7 weeks of age. Blood samples were obtained from the mice at 6 and 8 weeks of age to ascertain the immune response to these two antigens. At 10 weeks of age all mice were bled for total and differential white blood cell counts. The mice were then killed. Their spleens were weighed and a weighted portion of the organ was made into a single-cell suspension which was used to calculate the total number of nucleated spleen cells. The remainder of the spleen was fixed in formalin and examined histologically. The neck region was also examined grossly and histologically for thymus tissue. Neither gross nor microscopic thymus fragments were found in any of the thymectomized mice.

No statistically significant differences were found among the three groups of mice in the antibody response to BSA and ORBC, total lymphocytes in the peripheral blood, rate of weight gain, spleen weight or number of nucleated spleen cells. There were, however, highly significant differences ($P < 0.001$) between thymectomized treated and thymectomized untreated mice in both mortality and splenic lymphocyte depletion. When the experiment was terminated at 10 weeks of age, six of 14 thymectomized untreated mice had died with signs of wasting. None of 14 thymectomized treated mice had died or shown signs of wasting. Deficiency of small lymphocytes in the splenic follicles and marked reduction in germinal centers was observed in 7 of 14 thymectomized untreated mice. All 14 thymectomized treated mice, as well as the sham-thymectomized controls, had normal splenic architecture.

The ability of IgG derived from rabbit thymus or serum to prevent depletion of splenic lymphocytes in neonatally thymectomized mice was demonstrated in this experiment. However, the lack of immunologic impairment in the thymectomized mice used in these studies prevented any definitive measurement of the restorative ability of IgG. Thus, it is not possible at this time to state that IgG is the active principle elaborated by the thymus. The ability of neonatally thymectomized, IgG-treated, C₃H/He mice to reject an allogeneic tumor transplant is now being tested.

c. Induction of tolerance and immunity in mice newly born of tolerant mothers. Experiments conducted in this laboratory led to the prediction that offspring of immunologically tolerant mothers should be more susceptible than normal newborn animals to the induction of both tolerance and immunity (5). This prediction was verified in the experiments described below (6). Adult NIH female mice were immunologically paralyzed by injection of 500 mg of pneumococcal polysaccharide type III. The paralyzed mice were then bred and their newborn offspring were divided in groups and injected at birth with different quantities of polysaccharide. One week later the mice were challenged with 100 minimal lethal doses of Diplococcus pneumoniae type III. Mice that had become immune as a result of administration of polysaccharide survived the challenge, while mice that had been paralyzed by administration of polysaccharide did not. Thus, the paralyzing and immunizing doses of polysaccharide were determined. Similar determinations were made using newborn offspring from normal mothers. The results of the determinations are summarized in the following table:

Doses of Pneumococcal Polysaccharide Type III
Required to Paralyze and Immunize Newborn
Mice from Normal or Paralyzed Mothers

Status of mother	Micrograms of Polysaccharide required for	
	paralysis	immunity
Normal	0.5	0.05
Paralyzed	0.05	0.005

Thus, both the paralyzing and the immunizing doses of polysaccharide in mice newly born of paralyzed mothers were one-tenth of the corresponding doses for newborn offspring of normal mothers. The following set of statements is necessary in order to explain this result: (i) non-tolerant mice have in circulation natural antibodies specific for pneumococcal polysaccharide and transfer such antibodies to their offspring; (ii) tolerant (paralyzed) mice have no antibodies specific for the polysaccharide in circulation, and cannot transfer such antibodies to their offspring; (iii) as a consequence, the concentration of natural anti-polysaccharide antibodies is lower in offspring of paralyzed mothers than in offspring of normal mothers; (iv) the dose of antigen required to induce tolerance or immunity is a function of the concentration of natural antibodies specific for the antigen; (v) as a consequence, less polysaccharide is required to induce both tolerance and immunity in offspring of tolerant mothers than in offspring of normal mothers.

d. Detection and enumeration of cells producing antibodies against rabbit immunoglobulin G (IgG). The technique of localized hemolysis in gel (LHG) recently described by Jerne and co-workers (7) allows the enumeration of antibody-producing cells. The kinetics of antibody production can now be studied in terms of numbers of antibody-producing cells. However, the LHG technique is only applicable to cells producing antibodies against erythrocytic antigens, some bacterial polysaccharides (8) and certain haptens (9). With erythrocytic and bacterial antigens, small numbers of antibody-producing cells, as well as circulating antibodies, are found in unstimulated animals, making it doubtful that a true primary response can be studied. There is, therefore, a need for adaptation of the LHG technique to protein antigens.

Cells producing antibodies against rabbit IgG were detected with a technique devised in this laboratory during the past year (10). The technique involves plating of spleen cells from mice immunized with rabbit IgG in agar containing sheep erythrocytes sensitized with a non-hemolytic dose of the IgG fraction of commercial hemolysin. After incubation and addition of complement, round areas of hemolysis appear around spleen cells that release anti-rabbit IgG antibodies. The technique is based on the finding by Riha (11) that the hemolytic activity of IgG anti-erythrocyte antibodies could be increased by the addition of an antiserum specific for the IgG. It appears possible to employ the same procedure with IgG from species other than the rabbit.

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