Immunosuppressive effect of heterologous anti-immunoglobulin antisera in mice
by Dean David Manning

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in Microbiology
Montana State University
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Abstract:
Neonatal injection of mice with rabbit anti-μ antiserum has been shown to produce complete loss of direct and indirect plaque forming responses to sheep erythrocytes as well as loss of serum IgM and severe depressions of all other serum immunoglobulins. Similar injection of antiς1&sigma,2 anti&sigma1antibodies effects a loss of the indirect response but induces relatively minor alterations in serum Ig levels. Delaying initiation of anti-μ treatment until young adulthood results in a somewhat diminished effect on plaque forming responses and serum Ig levels but triggers the release of high serum levels of an aberrant μ-bearing protein.

Anti-μ and anti-ς1ς2 antisera, although profoundly affecting humoral antibody production, are not capable of altering the course of homograft rejection in mice.

Anti-μ suppression of genetically thymusless mice indicates that at least part of the target cells for suppression are bone marrow derived. A working model for the maturation of humoral antibody producing cell lines as it relates to these data is discussed.

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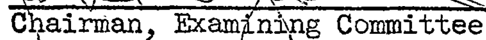
Approved:

Head, Major Department



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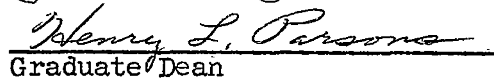

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ABSTRACT

Neonatal injection of mice with rabbit anti-μ antiserum has been shown to produce complete loss of direct and indirect plaque forming responses to sheep erythrocytes as well as loss of serum IgM and severe depressions of all other serum immunoglobulins. Similar injection of anti-$\delta_1\delta_2$ or anti-δ_1 antibodies effects a loss of the indirect response but induces relatively minor alterations in serum Ig levels. Delaying initiation of anti-μ treatment until young adulthood results in a somewhat diminished effect on plaque forming responses and serum Ig levels but triggers the release of high serum levels of an aberrant μ-bearing protein. Anti-μ and anti-$\delta_1\delta_2$ antisera, although profoundly affecting humoral antibody production, are not capable of altering the course of homograft rejection in mice.

Anti-μ suppression of genetically thymusless mice indicates that at least part of the target cells for suppression are bone marrow derived. A working model for the maturation of humoral antibody producing cell lines as it relates to these data is discussed.

INTRODUCTION

There is a rapidly growing body of evidence to establish the presence of immunoglobulins on the surface of lymphoid cells in mice (1-8). The classes of these immunoglobulins and their distribution, however, are still subjects of controversy. The immunoglobulins detectable on spleen cells, for example, have been variously reported as being distributed among all of the major classes, IgM, IgG, and IgA (2, 3, 5), and as being confined primarily to IgM (4, 6, 9). Some investigators (2,3) have detected only a single class of heavy chains on any given cell, whereas others (5, 8) have presented evidence for possible multiple heavy chain classes on each cell. It is generally agreed that many peripheral lymphoid cells, such as those in the spleen, bear surface immunoglobulins. There is conflicting evidence, however, as to whether cells of thymic origin are essentially lacking in these structures (2, 6) or bear them in low but detectable numbers (3, 7, 10).

Available evidence universally points to these surface immunoglobulins as products of the cells upon which they appear, and it is generally thought that these molecules all function somehow in antigen recognition. There is, however, some evidence that the antigen binding or "receptor" function may actually be limited to IgM (4, 11, 12).

Several approaches for determining the biological significance of these proteins have been based upon utilization of a wide

selection of antisera directed against whole immunoglobulins or their constituent chains. It has been shown, for example, that the plaque forming response of immune cells cultured *in vitro* can be suppressed by treatment with antisera against whole gamma globulin, IgM, IgG or κ light chains (13-16). It has also been reported that pretreatment of lymphoid cells with antibodies against gamma globulin can diminish the graft-vs-host (GVH) reaction when these cells are injected into appropriate hosts (17-19). Similar pretreatment experiments using various antisera of single chain specificity have demonstrated that suppression of the GVH reaction is possible only with anti-light chain (anti-κ) antibodies (11) and that adoptive antibody production can be prevented only with anti-light chain or anti-μ antibodies (12).

There have been relatively few attempts to alter the immune responses of intact animals with anti-immunoglobulin antisera. Antibodies against human myeloma λ-chains which cross-react with mouse κ-chains have been shown to inhibit the synthesis of certain κ determinants when injected into neonatal mice, but overall immunoglobulin levels were not affected (20). In chickens, the administration of anti-μ antibodies during embryonation together with bursectomy and additional anti-μ at hatching has produced agammaglobulinemia (21), apparently by plasma cell line elimination (22).

Our approach has been to treat neonatal or young adult mice with heterologous antisera against specific heavy chains and then assay these animals for suppressed immune responses, both humoral and cellular. We have previously reported that such neonatal treatment of mice with anti-μ produces absolute suppression of IgM production, as reflected in serum immunoglobulin level and direct plaque formation, and severe reduction of all other serum immunoglobulins (23). This agrees with a recent observation of decreased numbers of antibody forming cells in the spleen, lymph nodes and gut in germfree mice treated neonatally with anti-μ (24). We report here the extension of our studies to include mice treated initially as young adults, the effect of suppressive treatment on homograft rejection, and our investigations of the mechanism and extent of this type of suppression.

MATERIALS AND METHODS

Animals. BALB/c mice were used in all of these studies; for the target cell experiments, genetically thymusless (nude) mice of the strain described by Pantelouris (25) were also used. Because these nude mice cannot suckle their young, they must be obtained by mating animals heterozygous for the nude trait. The nude and phenotypically normal littermate progeny so derived were maintained on sterilized Purina 5010C feed and acidified-chlorinated water. BALB/c mice were weaned on day 24 and nude mice on day 30.

Preparation of Immunoglobulins. IgM, IgA, and IgG_2 were isolated from the sera of BALB/c mice bearing, respectively, the myeloma tumors MOPC 104-E (λ u), MOPC 406 (kα) and MOPC 173-D (kγ_2). IgG of both subclasses, $IgG_{1,2}$ (k$\lambda\gamma_1\gamma_2$), was prepared from normal BALB/c serum. Purification of IgM and IgA was achieved by successive treatments with Pevikon block electrophoresis, gel filtration on columns of Sephadex G-200 (Pharmacia Fine Chemicals, Rochester, Minn.) and DEAE-cellulose chromatography, using gradient elution (26). Purification of both types of IgG preparation included an initial precipitation step with ammonium sulfate (26), followed successively by treatments with DEAE-cellulose and Sephadex G-200. Purity of all preparations was checked by immunoelectrophoresis developed with high titered anti-whole mouse serum

and Ouchterlony gel diffusion developed with commercial (Meloy Laboratories, Springfield, Va.) class- or subclass-specific antisera.

Preparation of Antisera. Antisera were prepared by subcutaneous injection of rabbits. Each animal received two weekly injections of 10 to 15 mg of purified immunoglobulin in complete Freund's adjuvant (Difco Laboratories, Detroit, Mich.) and four to six further weekly injections of 10 to 15 mg in incomplete Freund's adjuvant (Difco), until serum precipitin titers ceased to rise. All antisera were routinely absorbed with BALB/c red blood cells, mouse α-globulin (Fraction IV, Pentex Biochemicals, Kankakee, Ill.) and mouse albumin (Fraction V, Pentex), although such specificities were generally lacking in the unabsorbed antisera. Light chain specificities were removed from the anti-IgM antiserum by absorption with IgA and $IgG_{1,2}$. Similarly, anti-$IgG_{1,2}$ antiserum was made $\gamma_1\gamma_2$-chain specific by absorption with IgA and IgM. A portion of the anti-$\gamma_1\gamma_2$ serum was further absorbed with IgG_2, rendering in anti-γ_1 specific. Absorptions with soluble proteins were made at 37 C for 1 hr, then overnight at 5 C, followed by centrifugation at 25,000 x G for 30 min. Multiple absorptions were made until no further precipitate could be detected, resulting in a slight final excess of absorbing protein. The unabsorbed anti-$IgG_{1,2}$,

used for facilitation of indirect plaques, showed no cross-reaction with IgM or IgA in immunoelectrophoresis. Specificity of all absorbed antisera was checked by immunoelectrophoresis and Ouchterlony gel diffusion using commercial (Meloy) class-specific antiserum standards. The only antiserum not showing complete heavy chain specificity by these tests was the anti-γ_1, which retained a slight trace of anti-γ_2 activity. Activities of antisera were evaluated in Ouchterlony gel diffusion plates. The antisera used for suppression were capable of producing precipitin bands at dilutions of 1:64 (anti-μ) or 1:128 (anti-$\gamma_1\gamma_2$ and anti-γ_1) when reacted against normal or myeloma mouse sera diluted 1:4.

Suppressive Treatment. Each litter of mice was divided between animals injected intraperitoneally with anti-immunoglobulin serum and controls similarly injected with normal rabbit serum. Neonatally treated animals were injected with 0.05 ml on the day of birth (day 0). The size of subsequent injections, given at two to seven day intervals, was increased slowly until the total desired dose was achieved. At no time did a single injection exceed 0.35 ml.

Immunization. Specific immunization was carried out by intraperitoneal injection of sheep red blood cells (SRBC) which had been washed three times and resuspended in phosphate-buffered