Low Molecular Weight Ia Antigens in Normal Mouse Serum

I. Detection and Production of a Xenogeneic Antiserum*

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Abstract. The observation that mouse serum can specifically inhibit cytotoxic Ia antisera indicates that substantial quantities of Ia antigen are present in normal mouse serum. The inhibitory substance in serum is dialyzable and is therefore probably of low molecular weight; it presumably represents a degraded form of cell-bound Ia antigen. A rabbit antiserum specific for murine Ia antigens was obtained by immunizing rabbits with mouse serum and then absorbing the resultant antiserum with dialyzed mouse serum. The binding of this antiserum to mouse leucocytes was detected by an indirect rosetting technique. The specificity of this antiserum was established as follows: (a) serum from mouse strains which possessed the corresponding Ia specificities absorbed out the antileucocyte antibodies; (b) binding of the rabbit antibody to leucocytes was inhibited by mouse anti-Ia sera but not by mouse antibodies against other regions of the H-2 complex; (c) conversely, binding of mouse cytotoxic Ia antibodies to leucocytes was specifically blocked by the rabbit antiserum; (d) antisera produced in rabbits against serum from recombinant mouse strains showed the correct Ia specificities. The rabbit anti-Ia serum was used to demonstrate that 90% of splenic B cells and 40 to 50% of splenic T cells are Ia +. In order of content of Ia + cells, the different lymphoid populations were ranked spleen > thymus > bone marrow > peripheral blood > lymph node.

Introduction

The immune responses to certain specific antigens are controlled by a group of immune response (Ir) genes linked to the major histocompatibility complex of the species (McDevitt and Benacerraf 1969, Benacerraf and McDevitt 1972). In mice, the Ir genes are associated with the H-2 complex. Using congenic and recombinant mouse strains, it has been possible to produce antisera which detect Ir region-associated (Ia) antigens (Sachs and Cone 1973, David et al. 1974, Hauptfeld et al. 1973, Götze et al. 1973, Hämmerling et al. 1974). Whether the Ia antigens are directly associated with the Ir gene products or play an unrelated functional role is at present a matter of controversy. In the hope

* Abbreviations used in this paper: Ir = immune response, Ia = I region-associated, FCA = Freund’s complete adjuvant, FIA = Freund’s incomplete adjuvant, FCS = fetal calf serum, PBS = Phosphate buffered saline, BSA = bovine serum albumin, SRBC = sheep red blood cells, Ig = immunoglobulin, CTU = cytotoxicity units.
of answering these questions we have attempted to purify and chemically characterize the Ia antigens.

This paper describes our initial experiments, which clearly demonstrate that substantial quantities of Ia antigen exist in normal mouse serum. The Ia antigen is of low molecular weight and probably represents a degraded form of the cell-bound antigen. Furthermore, by immunizing rabbits with mouse serum, a xenogeneic antiserum specific for murine Ia antigens was obtained.

Materials and Methods

Animals. A wide range of inbred mouse strains was used; the strains, together with the II-2 haplotype, H-2 region composition, and Ia specificities of each strain, are listed in Table 1. Xenogeneic antisera were produced in outbred New Zealand rabbits of either sex.

Antisera. Several mouse antisera directed against different mouse alloantigens were prepared (Table 2). The sera were produced by giving adult female mice a weekly intraperitoneal injection of a suspension of thymus, lymph nodes, and spleen for six weeks, and thereafter bleeding and immunizing on alternating weeks. The sera were pooled and stored at -70°C. Sera were also obtained from Dr. M. Cherry, Jackson Laboratory, Bar Harbor, Maine, under the auspices of a contract administered by the transplantation Immunology Branch, N.I.H., Bethesda, Maryland. These sera are described in the Catalogue of Mouse Alloantisera.

Antisera against mouse serum were produced by injecting rabbits in multiple sites with 0.5 ml of mouse serum emulsified in 0.5 ml of Freund’s complete adjuvant (FCA). Three to four weeks later the rabbits were challenged in a similar manner with 0.5 ml of mouse serum emulsified in 0.5 ml of Freund’s incomplete adjuvant (FIA). The animals were bled out 7 to 14 days after this last injection and the serum collected and stored at -20°C.

A specific xenogeneic anti-Ia serum was obtained by absorbing the rabbit antiserum with dialyzed mouse serum of the appropriate strain. To 1 ml of rabbit anti-CBA/H mouse serum was added 2 ml of exhaustively dialyzed CBA/H mouse serum (dialyzed for 4 days against 100 volumes of phosphate buffered saline (PBS) at 4°C, the dialyzate being changed every 24 hours) and 2 ml of PBS. The mixture was incubated at 4°C for 5 days and then spun at 100,000 g for 60 minutes at 4°C to precipitate any antigen-antibody complexes. The supernatant was collected and stored at -20°C.

Preparation of Cell Suspensions. Spleen, thymus, lymph node, and bone marrow cell suspensions of high viability (85 to 95%) were prepared as has been previously described (Parish et al. 1974). Briefly, the procedure consisted of preparing the cell suspensions at room temperature (15 to 25°C) and removing large aggregates and fine debris by multiple centrifugations of the cells at low g force. For cytotoxicity and rosetting assays, red cells were removed from spleen cell suspensions by exposing the cells to Tris-NH₄Cl or Ringer’s solution (Dain and Hall 1967). In addition, for cytotoxicity assays, dead cells were removed from spleen cell preparations by treating the cells with glucose-PBS and then filtering the cells through a small plug of cotton wool (Von Boehmer and Shortman 1973). In some cases, the preparations of spleen cells were treated with anti-Thy-1 (θ) serum and complement. This procedure has been reported elsewhere (Kirov 1974). Peripheral blood leucocytes were isolated from blood by centrifuging heparinized blood on Isopaque/Ficoll (Parish et al. 1974).

Cytotoxicity Testing. Lymphocytotoxicity tests were performed with spleen cells and rabbit complement. The complement was selected for its low toxicity, was not absorbed, and was used at a 1:12 dilution in medium L15 (Microbiological Associates, Baltimore, Maryland) containing 0.5% bovine serum albumin (BSA) (Miles Laboratories Inc., Kankakee, Illinois). Cell death was estimated by the trypan blue exclusion technique (Boyse et al. 1964). Cytotoxic tests were always performed as two-stage tests in microplates. Briefly, 50 μl of spleen cells (5 × 10⁶/ml in L15/0.5% BSA) were mixed with 50 μl dilutions of antiserum in each well of a microplate and incubated at room temperature for 10 minutes. The wells of the microplate were then flooded with medium, the cells were sedimented by centrifugation, and the supernatant was removed and replaced with 50 μl of complement. After 30 minutes at 37°C, the cells were again sedimented and the supernatant was removed and replaced by trypan blue. For each assay from 100 to 200 cells were scanned.