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Workshop 22

Allotypes. II

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The workshop focused largely on the use of allotypes as cell markers in studies of the immune response or in other biological investigations. The regulation of immunoglobulin production and the presence of immunoglobulins on cell surfaces were important topics.

The production of donor-type immunoglobulins of several classes was followed in the serum of sublethally irradiated allotype congenic mice which had received mouse spleen cells previously incubated for 2 hours with rabbit anti-μ sera. Mice receiving antibody-treated cells showed a 70–90% suppression of the development of donor IgG_{2a}, IgG_{2b}, and IgG_1 as compared to levels in mice receiving untreated cells. Mice receiving cells treated with anti-IgG were not suppressed. This suggests that

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there are μ receptors susceptible to suppression on the precursors of IgG-producing cells but gives no evidence for such susceptible γ receptors. Of course, this does not rule out γ receptors but means that, if they exist, they are not susceptible to suppression by incubation with antiserum. The data are consistent with other studies in embryonic chicks and newborn mice where injection of anti-μ antibodies can be effective in suppressing subsequent production of IgG antibodies. Since anti-μ was also shown to suppress adoptive secondary responses to sheep red blood cells and *Brucella,* resting B (memory) cells may also have susceptible μ receptors. In a very active discussion of allotype suppression in both mice and rabbits, data was presented suggesting that cells capable of producing the suppressed allotype are present in both maternally allotype suppressed and actively suppressed animals and can be stimulated by different procedures in the two types. Heterozygous Ig-1^a/Ig-1^b spleen cells suppressed for "b" allotype injected into irradiated Ig-1^a homozygous mice produce a burst of Ig-1^b globulin followed by reestablishment of a suprresssed state. This suppression was earlier shown to be T (thymus derived) cell mediated. Similar bursts of suppressed allotype production in heterozygous rabbits can be obtained by injection of rabbit antibody to the suppressed allotype. Peripheral blood lymphocytes from normal rabbits incubated *in vitro* with anti-b9 antibody give rise to much higher relative and absolute levels of b9 immunoglobulin when injected into lethally irradiated b4 rabbits. Immunofluorescent analysis also shows increased numbers of b9-producing cells in these recipients. The contrasting results obtained from anti-μ and antiallotype treatment were explained by the time differences implicit in the two techniques. Since the anti-μ treatment lasted only a short period *in vitro,* coated cells would be eliminated upon introduction into the recipients perhaps by opsonization or different cell migrations, whereas the 24 or more hours of anti-b9 left enough time for the involution of the b9–anti-b9 complexes on the cell surface. This involution and accumulation of complexes inside the cell might stimulate cell transformation, division, and differentiation leading to immunoglobulin production. These stimulated cells would be b9 committed, and would give increased b9 synthesis on cell transfer.

The question of allelic exclusion in all lymphoid cells, thought to

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be settled in the affirmative, was reopened by results derived from the mixed-antiglobulin (rosette) technique. In the presence of a mixture of antibodies to the b4 and b6 globulins, frozen peripheral blood lymphocytes from b4–6 heterozygous rabbits formed mixed rosettes with sheep cells which were coated with either b4 or b6 globulin. Controls show that artificial mixtures of b4–b6 and b6–b6 cells do not give mixed rosettes and that lymphocytes tested under a variety of conditions did not passively take up allotypic immunoglobulin. Twenty-day-old homozygous b4 offspring of 4–6 mothers did not give mixed rosettes even though they were bathed in maternal 4–6 globulin. In adult rabbits, often more than 50% of rosettes were mixed rosettes, and the proportion of lymphocytes forming rosettes was generally more than 30%. Young b4–6 rabbits had about 10% rosetting lymphocytes and mixed rosettes in heterozygotes were consistently found by about 12 weeks. In contrast, data derived from immunofluorescence and autoradiography show a low percentage of lymphocytes exhibiting both allotypes in heterozygotes. In addition, a rosette assay for studying human lymphocytes did not reveal cells expressing more than one immunoglobulin allele although only a few percent of rosettes were found altogether in this study. It is possible that the rosette technique which found mixed rosettes was more sensitive than other techniques, but more work will be done before the absolute generality of allelic exclusion in lmyphocytes can be determined.

Allotype systems in sheep, cattle, and chickens which were described offer valuable markers for studying the production of anti-viral antibodies in fetal lambs, concordance of allotypes in chimeric fraternal twins in cattle, or the loss of a serum protein possibly homologous to mammalian IgA in chickens with a rapidly growing transplantable lymphoid tumor. Immune responsiveness to IgA allotypes in mice is controlled by a gene which maps indistinguishably from Ir-1, a gene in the H-2 region which controls immune responsiveness to a number of different antigens.