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Studies on Immunological Tolerance to Soluble Proteins. I. Organ Distribution of Antigen in the Adult Mouse.* (32221)

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Following a neonatal injection of human gamma globulin (HGG) mice are rendered tolerant for at least 14 weeks(1). This state of immunological unresponsiveness has been thought to depend upon an interaction between the antigen and the early cell forms responsible for specific immune competence (2). Other studies have indicated the possibility of an extracellular regulatory mechanism for antibody synthesis(3). A specific state of immunological tolerance also can be produced in adult animals. HGG has been found to render adult C57 Bl/6 mice tolerant after a single inoculation of a relatively small dose of protein antigen(4).

Mice tolerant to HGG do not recognize HGG as a foreign material as shown by the exponential elimination of the human globulin from the mouse sera(1). The specific failure of unresponsive mice to form circulating antibodies may represent a failure of antigen uptake by the macrophages. A better understanding of the central problem of immunology, the mechanism of the immune response, can be gained by a better knowledge of the cellular basis for distinguishing between self and non-self. It seems that the degree of immunogenicity correlates with antigen localization(5).

The purpose of this investigation was to study the fate of the tolerance-inducing antigen in adult mice under different experimental conditions. The organ distribution of a low amount of antigen, known to produce specific immunological unresponsiveness, was studied in groups of previously untreated, tolerant, actively immunized and passively immunized mice.

Materials and methods. Animals: Inbred HS Swiss albino mice were obtained from Dr. B. R. Jennings(1). They were fed with a balanced diet and KI was added to the water bottles.

Antigens: Human gamma globulin (HGG) obtained from Pentex, Inc., Kankakee, Ill.,

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Lot No. 27 was centrifuged for 30 minutes at 40,000 × *g* prior to use. Conjugation of HGG with I^{131} was done according to the technique by Rosen(6).

Radioactivity assay of organs: The radioactivity of each organ study was measured by direct organ counting using a Well Scintillation Detector DS-202 (V), Nuclear-Chicago. Values obtained represent the mean I^{131} count of a given organ from each subgroup in a time unit. All counts were performed simultaneously to avoid correction due to atomic decay.

Experimental: Four groups of 12 adult mice each were studied. At age 9 weeks, mice in all 4 groups (previously untreated, passively immunized, actively immunized, tolerant) were identically treated with a single intraperitoneal (IP) injection of 0.2 mg of centrifuged HGG-I^{131}. One group of mice, the previously untreated, remained as non-treated control until this time. Another group of mice was passively immunized, 12 hours prior to HGG-I^{131} treatment, with an IP injection of 0.1 ml of rabbit anti-HGG antiserum (Hyland Laboratories, Lot No. RP5-66). A third group was actively immunized at age 7 weeks (14 days prior to HGG-I^{131} treatment) by administration of a sensitizing subcutaneous injection of HGG in incomplete adjuvant; this consisted of 0.1 ml per mouse of a water in oil (w-o) emulsion containing equal parts of an aqueous suspension of HGG (16 mg/ml) and Bayol F (Esso, Batch 322). The fourth group was rendered tolerant at age 7 weeks by an IP injection of 0.2 mg of centrifuged HGG. This procedure has been found in this laboratory to induce specific immunological tolerance in HS Swiss mice(7).

The mice were killed by pulling the necks at 4, 8, 24, and 48 hours after HGG-I^{131} administration, thus forming 4 subgroups of 3 animals in each numbered group. They were frozen immediately after being sacrificed, until the I^{131} assay was performed, 4 hours after the freezing of the last subgroup. Freezing the animals proved satisfactory for preserving the organs. Assay for radioactivity was carried out separately on the following organs: heart, lungs, thymus, kidney, spleen, adrenal glands, liver, bone, inguinal and axillary lymph nodes, brain and blood.

Results. Following a single inoculation of a small tolerance-inducing dose of HGG-I^{131}, a characteristic distribution pattern was found among the organs of adult mice. The liver showed the largest absolute I^{131} count in all 4 groups, the highest being in the animals passively immunized with heterologous antiserum. This is to be expected since the liver constitutes approximately 7% of the total body weight of a normal mouse of 20-25 g. However, the localization of antigen in the bone marrow (femur), brain, and adrenal gland, as interpreted by their respective I^{131} activities, was either consistently low or highly irregular and inconclusive. These 3 tissues, therefore, were left out of Fig. 1, which illustrates the mean labeled antigen uptake, expressed per unit weight of organ in the 4-, 8-, 24- and 48-hour subgroups.

The highest I^{131} count per 100 mg of tissue was found in the lymph nodes of all 4 groups (Fig. 1). Four hours after HGG-I^{131} administration, the lymph node activity of the sensitized mice was twice as high, and in the passively immunized mice 3 times as high, as that of the previously untreated group; moreover, at that time tolerant mice had I^{131} count one-sixth that of passively immunized and one-fifth that of actively sensitized mice. This pattern was not observed at the 8- and 24-hour determination, by which times the tolerant mice had the highest lymph node activity. This activity dropped again in this group by 48 hours.

The pattern of activity displayed by the thymus parallels in some respect that of the lymph nodes, but the antigen concentration of this organ was, in all 4 groups, approximately a fifth that of the lymph nodes. The thymus of both groups of sensitized mice had 4-hour counts at least 2 and 3 times as high as those of the tolerant and untreated mice, respectively. By 48 hours the tolerant mice showed the highest count.

Tolerant and previously untreated mice showed no significant differences between the antigen uptake of their respective organs, except for the thymus at 4 hours. More-

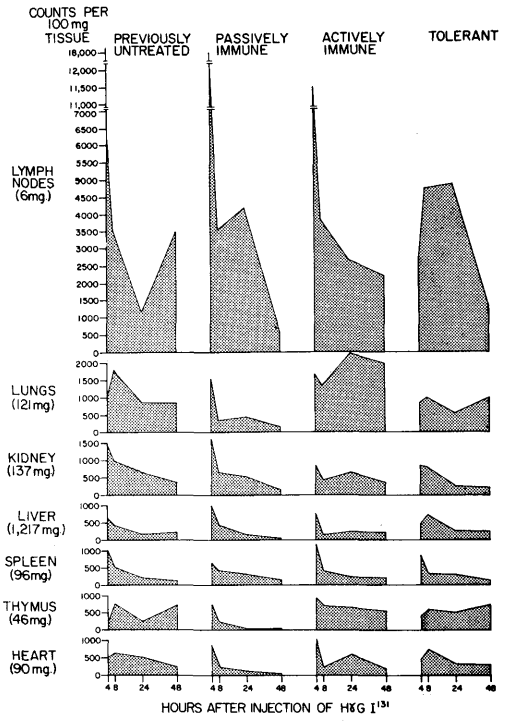

FIG. 1. Mean antigen concentration (expressed as counts per 100 mg of tissue) in various organs at 4 times interval after administration. Average organ weights are in parentheses. Values represent left kidney and right inguinal lymph node only.

over, passively and actively immunized mice were not significantly different in antigen uptake of their respective organs, except for the spleen, which had a lower uptake in the passively immunized mice. The 2 immunized groups both had a higher antigen uptake than that of either of the non-immune groups, particularly with respect to their lymph nodes and thymus. An exception to this generalization was, again, the spleen of the passively immunized group.

Because of the similar patterns of the 2 immunized groups, it is thought that the heterologous antiserum used to render mice passively immune did not interfere with antigen localization. Differences found are considered to be due to the type of immunity induced.

Discussion. This investigation was undertaken to evaluate the fate of a relatively small amount of antigen known to produce immunological tolerance in the adult mouse (4-7). Previous studies on organ localization of antigen deal mainly with neonatally induced tolerance(5,8). The present report is a study of the immediate distribution of a small amount of tolerance-inducing antigen in 4 groups of adult mice under various experimental conditions. Roberts has demonstrated the significance of the interaction between antigen and immunologically competent cells, the first few hours following the primary immune stimulation(9). Data presented here certainly agree with the postulate that discrimination between self and non-self takes place immediately after contact with the foreign substance.

The question of prime consideration was if previously untreated adult mice, injected with a small amount of aggregate-free HGG, handled antigen in a fashion similar to previously immunized mice. Campbell and Garvey(10) have shown that immunized animals had their highest antigen concentration in the liver. Fig. 1 indicates similar high values for both actively and passively immunized mice. Previously untreated and tolerant mice, on the other hand, had a significantly lower antigen uptake in all organs. This response indicates a shortage of organ localization. Fig. 1 clearly shows the low radioactivity, displayed particularly by the lymphoid tissue, of untreated mice organs compared to passively immunized mice organs. This similar pattern of previously untreated and tolerant mice further indicates their state of unresponsiveness. On the other hand, passively immunized and actively immunized mice can be classified as immune.

The efficacy of rabbit antisera passively administered into tolerant mice was demonstrated by Hemphill *et al*(11). In the present investigation the highest radioactivity in the lymph nodes, kidney and liver was found in the passively immunized mice, while the highest activity in the spleen and thymus was found in the actively sensitized animals. It is tempting to elaborate on the significance of these variables. It may very well be that active immunization with cell proliferation gave the higher radioactivity in the spleen and thymus, while passively sensitized animals with formation of antigen-antibody

complexes had their greater activity in the liver and kidney.

It was also considered whether or not normal adult mice receiving a non-immunizing dose of antigen respond in a manner similar to newborn tolerant rats. Insufficient data referring exclusively to the fate of antigen in the newborn rat during the first 48 hours makes a complete analysis of the differences difficult. It would seem by the works of Mitchell and Nossal(12) that neonatally treated rats have an initially low concentration of bovine serum albumin in the spleen and thymus. This is followed by an increased antigen localization at 24 hours, which begins decreasing by 48 hours. A similar pattern was found in this study for the thymus of adult tolerant mice in previously untreated and tolerant groups and lymph nodes in tolerant mice. Somewhat different was the uptake of HGG-I^{131} by lymph nodes of previously untreated mice.

Variation found in organ radioactivity among the animals forming each subgroup was, with one exception, smaller than 20% of the total subcount. Low deviation was also found by Hale and Stoner(13) and Jennings (14) in previous studies on the antibody synthesis of this highly inbred strain of Swiss mice. The findings reported can be readily explained by an extracellular mechanism controlling antibody formation(3), but persistent concentration of antigen and ineffective phagocytosis can also result in inhibition of a specific clone of cells(2), as is postulated by followers of the theory of intracellular control of antibody synthesis.

Immunological unresponsiveness can follow a blockage of the immune process at different levels. Levine and Benacerraf(15) found that responsive and non-responsive guinea pigs break down antigen in a similar fashion. Unresponsiveness was thought to be a genetic failure to form specific antibodies. Data here presented, that a tolerance-inducing dose of antigen is followed by a shortage of antigen localization, indicate that this phenomenon was caused by a block in the immune response at an early stage, prior to antigen digestion.

Summary. Organ localization of antigen was studied in adult mice. A small tolerance-inducing dose of radiolabeled protein was given to each animal in 4 experimental groups of mice. Radioactivity as an expression of organ distribution of the HGG-I^{131} was determined at 4, 8, 24, and 48 hours following HGG-I^{131} administration. Blood, bone, brain, lymph nodes, thymus, spleen, liver, kidney, heart, lung and adrenal gland were assayed. A shortage of localization of antigen was found in the lymphoid tissues of tolerant and previously untreated mice. The liver was found to be the organ with the single highest radioactivity in all groups. Lymph nodes showed the highest activity per gram of organ weight. This was primarily significant for sensitized mice. The pattern of antigen distribution in the tolerant animals was interpreted as a failure, early in the immune response, to recognize the foreign material as non-self.

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