Subpopulations of T-lymphocytes in Peyer's patches: Sensitivity to antilymphocyte serum and adult thymectomy[1]

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Summary. Anti-lymphocyte (ALS) treatment or adult thymectomy of the donor have been shown to depress respectively the cell proliferation and the cytotoxicity in the graft-versus-host (GVH) reaction. A quantitative assay and the histological criteria of the GVH reaction have been used to demonstrate that all the known subpopulations of T-lymphocytes involved in the GVH reaction are present in the Peyer's patches as well as in the spleen and mesenteric lymph nodes in the rat.

The presence of T-cells within the Peyer's patches was demonstrated by the use of T-cell markers[2–5], the study of migratory patterns of thymocytes in the small bowel[6,7] and functional tests such as in vitro culture with specific mitogens[8], the mixed lymphocyte reaction[9] and the graft versus host (GVH) reactivity[9–11]. The latter immune response has been shown to be mediated by an interaction among different subpopulations of T-lymphocytes[12,13] with different sensitivities to corticosteroid treatment, antilymphocyte serum or adult thymectomy[14,15]. In a previous report we have demonstrated the presence of cortisone sensitive T-cells within the Peyer's patches, as well as in the spleen and mesenteric lymph nodes in the rat[16]. The aim of the present study was to test in the Peyer's patches the presence of T-cells sensitive to antilymphocyte serum (ALS) or adult thymectomy (ATx). Any activity of such cells, if present, was compared with those of the spleen, thymus and lymph nodes using clinical and histological criteria of the GVH reaction and a quantitative assay, the paravascular infiltration of the liver.

Material and methods. According to a previously described procedure[10], lymphoid tissues were implanted into the anterior hepatic lobe of F1 Hybrid rats (Aug. x Lew). The Peyer's patches, the spleen, the mesenteric lymph nodes and the thymus were transplanted according

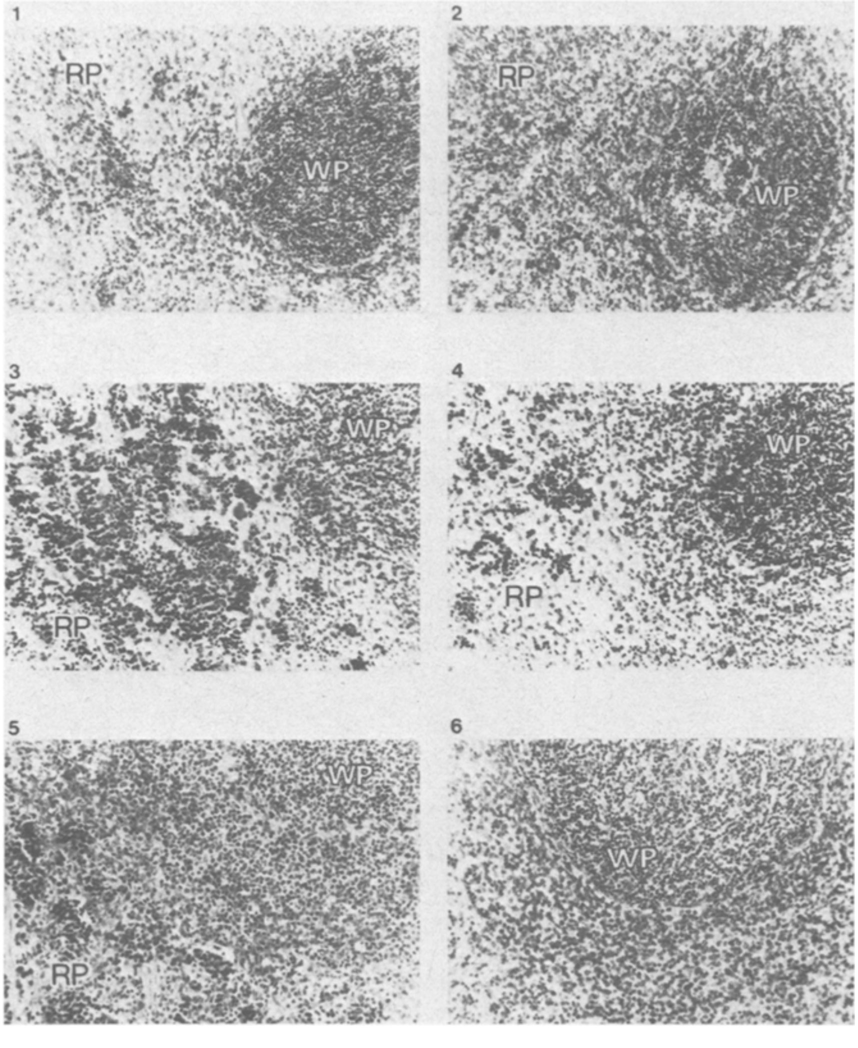

Fig. 1. Spleen section of the recipient (hematoxylin-eosin ×65.) *1* 8 days after implantation of ATx semi-allogeneic lymph nodes (group d). *2* 8 days after implantation of ATx semi-allogeneic Peyer's patches (group d). In both cases no cellular depletion in the white pulp (WP) nor blastic infiltration in the red pulp (RP). *3* 15 days after implantation of ATx semi-allogeneic lymph nodes (group d). Diffuse cellular infiltration in the red pulp. *4* 15 days after implantation of ATx semi-allogeneic Peyer's patches (group d). Foci of cellular infiltration in the red pulp. *5* 8 days after implantation of ALST semi-allogeneic Peyer's patches (group c). *6* 8 days after implantation of ALST semi-allogeneic spleen (group c). In *5* and *6* characteristic cellular depletion of the white pulp and slight infiltration of the red pulp.

to 4 experimental schedules: a) Hybrid lymphoid tissues in F1 Hybrid recipients (50 animals), b) parental August lymphoid tissues in F1 Hybrid recipients (55 animals), c) ALS-treated (ALST) parental August lymphoid tissues in F1 Hybrid recipients (60 animals), d) parental August lymphoid tissues (exept thymus) from adult thymectomized (ATx) donors in F1 Hybrid recipients (45 animals). In the group c the donor animals received an i.p. injection (1 ml) of rabbit anti-(rat-lymphocyte) serum (Microbiological Associates Inc., Bethesda, Maryland, USA, Lot No. 15071) 48 and 24 h before sacrifice. Thymectomy was surgically performed on August adult rat (weighing more than 200 g) 10 weeks before the removal of the lymphoid tissues for subsequent transplantation. For the nontreated (NT) donors, the mean number of lymphoid cells used for each implantation exceeded 10^7 cells. ALS treatment or adult thymectomy of the donor reduced slightly the number of the transplanted lymphoid cells which still exceeded 10^7 cells.

All recipients were examined daily for weight change and general appearance. They were killed by ether in groups of 5 postoperatively on days 8, 15 and 30. The spleen and liver were removed for histological studies and the extent of the paravascular infiltrates (PVI) within the liver was estimated in the non-implanted hepatic lobes, according to Bain's criteria[17].

Results. In control animals with an isogeneic implantation of lymphoid tissue, no clinical and no histological changes were observed. The hepatic lobes contained a similar number of PVI, whether they had been the site of [illegible]tation or not: this averaged 0.1/10 mm² [illegible] section in all the postoperative examinations from [illegible] days. When F1 Hybrids were implanted with NT-par[illegible] lymphoid tissues, no lethal GVH syndrome was observed whichever tissue was used. The clinical course of experimental animals with ALST Peyer's patches or Peyer's patches from ATx donors was similar to that observed after implantation of NT Peyer's patches. No postoperative mortality related to a GVH disease was observed, whichever tissue and/or treatment were used.

Histological examination of the spleen sections after implantation of NT parental Peyer's patches, lymph nodes or spleen (group b) showed cellular depletion in the thymus-dependent area of the follicle and blastic proliferation within the red pulp at the 8th, 15th and 30th postoperative days. At each postoperative time, adult thymectomy of the donor reduced markedly the cellular depletion of the white pulp after spleen, lymph nodes and Peyer's patches implantation. The cellular proliferation of the red pulp was depressed after each kind of implantation in group d at the 8th and 30th day but was still observed at the 15th day (figure 1). On the contrary, ALS treatment of the donor did not suppress the classical cellular depletion of the thymus-dependent area of the follicle, but reduced the cellular infiltration of the red pulp (figure 1). These features were observed after ALST spleen or lymph nodes implantation as well as after ALST Peyer's patches implantation.

Comparison of the PVI between the recipients of b, c and d groups is illustrated in figure 2. ALS treatment (group c) reduced significantly the extent of the paravascular infiltration in the liver. Between the 8th and 30th day, there was no change in the PVI count after ALST lymphoid tissue implantation. Similarly the lymphoid tissues from ATx donors induced a lesser paravascular infiltration in the liver of recipients, as compared with NT lymphoid tissues, but a significant increase in the PVI count was observed at the 15th postoperative day after implantation of spleen ($p < 0.01$) lymph nodes ($p < 0.05$) or Peyer's patches ($p < 0.05$) in this experimental d group. The numbers of PVI produced by the tested lymphoid tissues from treated animals were compared at the different postoperative times. After ALS treatment of the donor (group c), the PVI counts observed in the recipients of Peyer's patches, spleen, lymph nodes and thymus were not significantly different and they did not differ significantly from zero. After adult thymectomy of the donor (group d), no significant difference in the PVI count was observed at the 8th and 30th post operative day between the different implanted lymphoid tis-

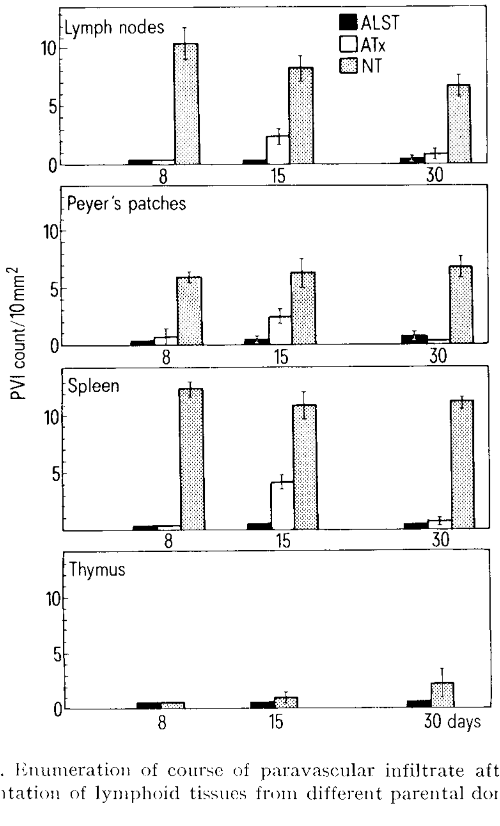

Fig. 2. Enumeration of course of paravascular infiltrate after the implantation of lymphoid tissues from different parental donors.

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sues, and they were not significantly different from zero. However, at the 15th postoperative day, the PVI count was increased and differed significantly from zero, but was of the same extent whichever lymphoid tissue was implanted.

The analysis of the different pretreatment of the donors demonstrated that ALS as well as adult thymectomy depressed considerably the PVI-forming capacity of the 4 lymphoid tissues tested in the experimental groups. At the 8th and 30th postoperative days, no significant difference between the two kinds of pretreatment was observed for each tissue under study. However, it must be noted that at the 15th day the Peyer's patches as well as the spleen or lymph nodes from ATx donors induced an hepatic paravascular infiltration of lesser importance than in control animals but significantly higher than after ALS treatment ($p < 0.05$). Thus, based on this criterion, it is suggested that T-lymphocytes present in the Peyer's patches have a reponse to ALS treatment and adult thymectomy closely related to that of other peripheral T-lymphocytes.

Discussion. T-lymphocytes subpopulations have been recently reviewed by Cantor and Weissman[18]. One class of them (T1) is sensitive to adult thymectomy, relatively insensitive to ALS and homes preferentially to the spleen. A second subpopulation (T2) is relatively insensitive to adult thymectomy, sensitive to ALS treatment and is found in highest concentration in lymph nodes, thoracic duct and blood. In the GVH reaction, the T1 lymphocytes are responsible for cytotoxicity whereas T2-lymphocytes act as 'amplifier' cells responsible for the cell proliferation[12]. The results reported above confirm the sensitivity of the T-cell subpopulations of the peripheral lymphoid tissues to ALS treatment and adult thymectomy. However, the relative proportion of these 2 lymphocytes subsets cannot be evaluated adequately with the experimental model used in this study. In fact, the GVH reaction observed after intrahepatic lymphoid tissue implantation is modulated by the interaction of a suppressor factor, probably the T-suppressor cell, to which the high values of PVI in non-treated F1 Hybrid recipients (group b) might be related[19,20]. The delayed increase (on the 15th day) of the PVI formation in the recipients of ATx lymph nodes spleen and Peyer's patches is in keeping with the previous finding that thymectomy in the adult life caused only a partial loss of capacity to develop suppressive activity[21]. The data reported in this study demonstrated that the T-lymphocytes of the Peyer's patches exhibited the same sensitivity to ALS or ATx treatment as those present in other peripheral lymphoid tissues. Thus it is concluded that all the known subpopulations of T-lymphocytes acting in the GVH reaction are present in Peyer's patches.

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Effects and duration of resistance acquired by rabbits on feeding and egg laying in Ixodes ricinus L.[1]

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Summary. Rabbits gradually developed a resistance against Ixodes ricinus ticks as a result of sequential experimental infestations. The resistance was characterized by an increased duration of feeding, by ticks engorging to a smaller extent and by reduced egg production. Once established, the immunity persisted for at least 9 months. Changes in the titre of circulating anti-I. ricinus antibodies was measured by indirect immunofluorescence.

Although the ecology, behaviour and epidemiology of I. ricinus have been extensively studied, the immunological reactions of the host to the bite of this tick are very poorly understood. In this work, the development, effects and duration of resistance acquired by the rabbit on feeding, egg production and hence fecundity are described. The occurrence of specific anti-I. ricinus saliva antibodies was also examined.

Materials and methods. To study the development of acquired resistance, 5 male rabbits (Himalayan breed, genotype aac^Hc^H), weighing 2 kg, were infested 4 times, each with 10 ♀♀ I. ricinus on days 0, 21, 49 and 84 of the experiment. The duration of immunity was investigated by re-infesting 2 of these animals 56 days after the 4th infestation, 2 others 183 days and the last rabbit 269 days after. The ♀♀ ticks, captured in the field on a 'blanket', were held at high humidity ($RH > 90\%$) and allowed to engorge along with ♂♂. It is known that fertilization is necessary for the final phase of feeding in ♀♀[2]. In order to avoid local cutaneous lesions impeding attachment, the ears were infested in turn, sometimes also the animals backs were used.

The ticks were protected by a nylon sheath, fixed at its extremities with an adhesive band; a collar prevented the rabbit from scratching. At the end of feeding, the ♀♀ I ricinus. (the male does not feed) detached from the host and were collected by opening the sheath. They were weighed and placed in a humid environment ($RH > 90\%$) for egg laying and hatching. The presence of circulating, specific anti-I. ricinus saliva antibodies were examined by indirect immunofluorescence, according to the techniques described elsewhere[3]. Salivary glands of ♀♀ ixodids, taken on the 4th day of feeding, were used as antigen. At this stage of engorgement of the ticks, the salivary glands contain a large number of antigenic acini. The threshold of specificity of the reaction was fixed experimentally at $^1/_{20}$.

Results. 1. Effects of acquired resistance in rabbits on the nutrition of ♀♀ I. ricinus. Preliminary note. During the 4 experimental infestations, all the ♀♀ I. ricinus attached; also most fed. However, on the 4th infestation 36.0% died on the host without engorging. In the text, the weight of fasting ♀♀ I. ricinus (which the mean weight is

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