Beta 1C and Immunoglobulins G and M in the Development of Rabbit Gut-Associated Lymphoid Tissues[1] (35060)

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Previously, we have described the significance of the rabbbit appendix, Sacculus rotundus and Peyer's patches (gut-associated lymphoid tissuse, GALT) for the immunological development and function of the animal (1–3). We also reported on the presence of immunoglobulins, antigens, antibody and β1C in follicles of the rabbit peripheral lymphoid tissues (PLT) (4). The term PLT is used to characterize those lymphoid organs (lymph nodes and spleen) that are dependent for lymphoid development on central lymphoid organs, the thymus and bursa or bursa-equivalent. We now report studies on the presence of β1C, IgM, and IgG in the developing rabbit GALT. In contrast to mesenteric and popliteal lymph nodes, follicles of GALT appear to bind minute amounts of β1C in the early stages of development only; in the mature organ, little or no evidence for presence of β1C is found. Furthermore, β1C, IgG, and IgM are present in GALT before they are found anywhere else in the lymphoid system.

Methods. New Zealand white male rabbits were obtained from a local breeder. Groups of 6–14 rabbits each were studied in the neonatal period, and at the ages of 1, 2, 3, and 4 weeks, and 3, 5, 12, 24, and 48 months. Histologic and immunofluorescent techniques were used as described earlier (4).

Results. The GALT of 1-day-old rabbits had little or no follicular development and no evidence for the presence of β1C, IgM, and IgG was obtained. At 1 week of age, the follicles of GALT were still poorly developed without clear differentiation between cortex and medulla. Few granules of β1C were noted in the center of these follicles, but many cells positive for IgG and IgM were found. The GALT of 2-, 3-, and 4-week-old rabbits showed well-developed follicles with a distinct cortex and medulla, and it was possible to localize a small amount of β1C in the medulla as bright granules (Fig. 1). In older animals β1C component of complement was not present in appendiceal follicles or, if occasionally present, was shown mainly in the upper parts of the follicles in the form of very scarce and small granules. From the age of 2 weeks on, many cells in the medulla of the follicles were strongly positive for IgG and IgM. Incubation of unfixed sections of appendix for 2.5 hr in citrate buffered saline, pH 3.3, resulted in only slight diminition of IgG and IgM content of the follicular cells. The same appendiceal follicles which showed almost complete absence of β1C in fresh frozen tissues were strongly positive for β1C if they had been incubated previously with fresh human serum or fresh rabbit serum (Fig. 2). Sections incubated with heat-inactivated serum remained persistently negative. In peripheral lymph nodes, no cells positive for β1C, IgG and IgM were present before the

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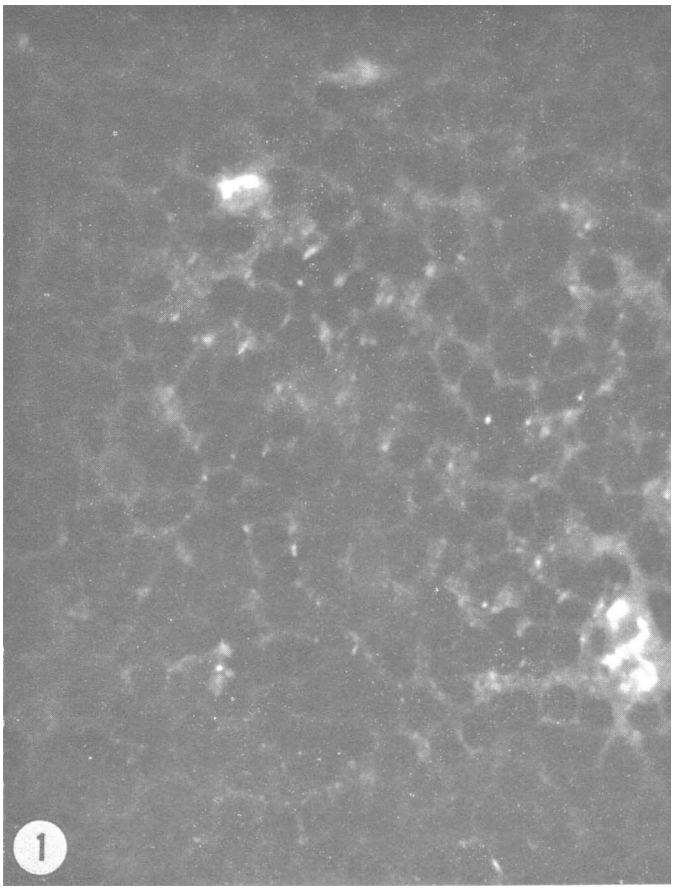

FIG. 1. Fixation of β1C in a fresh frozen section of appendiceal follicular medulla (4-week-old rabbit). Only a few positive granules are seen. Note two macrophages which showed orange autofluorescence of cytoplasma in the original preparation; × 480.

age of 2 weeks; from that age on their number increased greatly in lymph nodes and spleen. As noted earlier (4), germinal centers of PLT, when positive in direct staining for rabbit β1C, were also capable of fixing additional complement when incubated with heterologous (human) complement. Germinal centers negative for β1C and immunoglobulins G and M also did not fix heterologous or autologous β1C. By staining serial sections of GLT with antirabbit fibrin antiserum, a dense network of large and small vessels containing fibrin was seen around the lymphoid follicles. By this technique, few vessels were demonstrated in the follicular medulla.

Discussion. We have documented that cells positive for IgG and IgM appear in the GALT of the rabbit at a time when the peripheral lymph nodes have already been populated by thymus-derived lymphocytes (5) but do not yet contain immunoglobulin producing cells. The spleen at this time contains very few lymphoid elements. This observation supports our original contention (2, 6) that in rabbits, at least, immunoglobulin, and probably also antibody formation, takes its initial impetus from the gut-associated lymphoid tissues: appendix, sacculus, and Peyer's patches.

Furthermore, we have shown a striking difference in the capacity to fix β1C component of complement in PLT and GALT in fresh frozen tissues, despite the fact that both contain IgG and IgM and can fix β1C after *in*

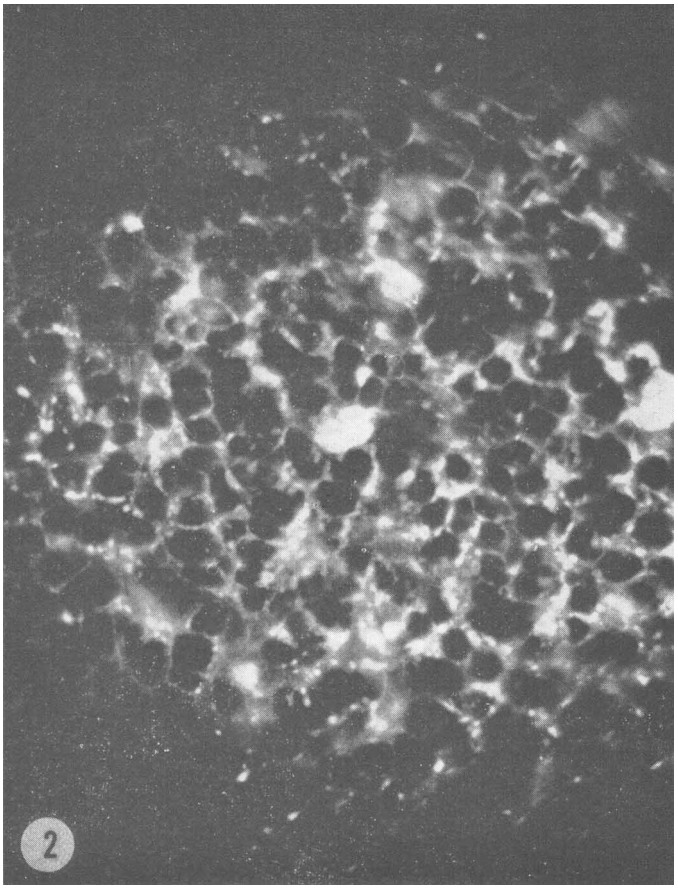

FIG. 2. Fixation of β1C in serial section of the same appendix as on Fig. 1 after incubation with fresh human serum for ½ hr; × 480.

vitro incubation with fresh sera.

The diminished or absent binding of β1C in follicles of GALT most likely is not an artifact. The specificity of our antirabbit β1C antiserum has been demonstrated by immunodiffusion and absorption techniques (4). In addition, mesenteric lymph nodes, strongly positive for β1C always served as controls for negative staining of appendiceal follicles. No positive staining for β1C was obtained when serial sections were incubated with inactivated human or rabbit serum. The possibility of secondary aggregation of immunoglobulins during our fixation procedure (and, therefore, stronger fixation of β1C *in vitro*) can also be ruled out because the same intensity of β1C staining was observed in acetone-fixed and fresh-unfixed sections. An antigen–antibody ratio not suitable for the fixation of β1C is improbable since strong staining for β1C was obtained after incubation with fresh complement *in vitro* of serial sections from the same tissues which were negative when stained with antirabbit β1C directly. Complement is not fixed in either acid and alkaline milieu (pH$>$8.5 and $<$6.8) but such extremes of pH are unlikely to be present in GALT *in vivo*.

Two possibilities to explain our findings have to be considered: (i) The supply of β1C *in vivo* is inadequate in the medulla of rabbit appendiceal lymphoid follicles, or (ii) there is an inhibitor mechanism influencing β1C binding *in vivo* in gut lymphoid tissue.

The vascular supply of the follicular medulla appears to be not so rich as that of

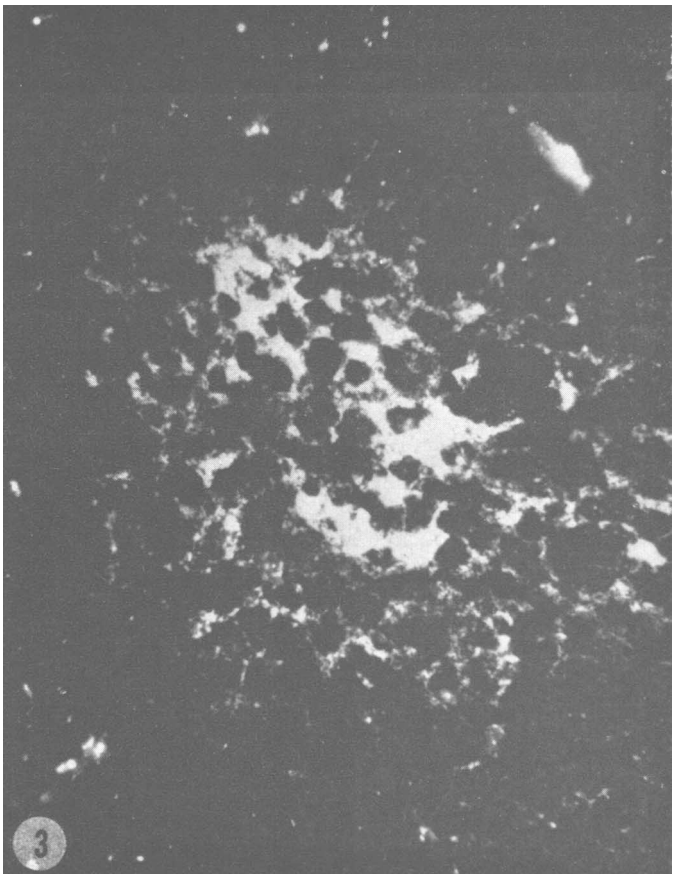

FIG. 3. Fixation of β1C in a germinal center of the mesenteric lymph node of the same rabbit; × 480.

germinal centers in peripheral lymph nodes (4, 7, 8). The latter are well supplied with numerous capillary branches which penetrate into germinal centers (9). The vascular supply of the follicular cortex of GALT is provided by numerous branches of the perifollicular arteries (7, 8, 10). But only a few of these branches penetrate into the medulla, and therefore, the amount of several complement components available at this site may be minimal.

This would explain why some β1C is found in early developing follicles only. These are small, do not have a large well-developed medulla as yet, and may, because of their size, have a better vascular supply than mature follicles.

The lack of β1C in the medulla of follicles which contain large amounts of immunoglobulins may also be explained by the activity of inhibiting substances which may be diminished during the freezing procedure, thus permitting the *in vitro* binding which we have demonstrated.

The most likely explanation of our findings seems to be that β1C does not reach the follicular medulla in the GALT. In follicles of peripheral lymphoid tissues, binding of complement may only be needed in the stage of rapid expansion of the germinal center after antigen contact and may be facilitated by the excellent vascular supply. The follicles of GALT differ considerably from the lymph node follicles. In the cortex of appendiceal follicles precursor cells proliferate rapidly without production of immunoglobulin (3, 12); binding of β1C would, therefore, not be expected. In the relatively avas-

cular "hollow" center of the follicle, the medullary cells differentiate to immunoglobulin-producing cells, and from this site populate other lymphoid tissues (11, 12): for these processes, binding of β1C may not be necessary.

Although our findings deserve much further study, they give additional evidence of striking differences that exist between the peripheral and gut-associated central lymphoid organs in the rabbit.

Summary. IgG and IgM are found in the rabbit gut-associated lymphoid tissues before they are found in peripheral lymphoid tissues. β1C is present in very early stages of development only. The implications of these findings are discussed.

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