Involvement of Thymus in Immune Response of Rabbits to Somatic Polysaccharides of Gram-Negative Bacteria

Abstract. *Plaque-forming cells elaborating antibody specific for* Salmonella enteritidis *somatic antigen have been demonstrated in the thymus of rabbits 5 days after a single systemic injection of a minute amount of this bacterial polysaccharide. A marked decrease in organ weight and loss of thymic cellularity was also seen, and was most pronounced at the time when antibody-forming cells made their appearance.*

The thymus is now recognized as playing a vital role in the development of the immunologic capabilities of the host, in that lymphoid structures in the embryo and the neonate appear to be populated with lymphocytes arising in the thymus (*1*). As the progeny of the thymocytes, small lymphocytes have been increasingly suspected of playing a role in the primary immune response, especially since they are known to be capable of developing into large pyroninophilic cells (*2*) and to recycle extensively from blood through the lymphatics and lymphoid structures (*3*). However, in the mature host the thymus itself, unlike all other lymphoid organs, has not been found to participate in the synthesis of antibodies after antigenic stimulation by way of the usual routes (*4*). It has been possible to evoke an immune response in the thymus only by injection of antigen directly into that organ (*5*) or into the mediastinum (*6*). In general, the failure of the intact thymus to respond to systemically administered antigen has been viewed as a consequence of a blood-thymus barrier that effectively excludes the penetration of antigen (*7*).

During an investigation of the cellular aspects of the immune response of the rabbit to the somatic polysaccharides of gram-negative bacteria (*8*), a single stimulus with *Salmonella enteritidis* somatic antigen (*9*) gave rise to large numbers of antibody-forming cells; most of these cells were found in the spleen, but significant numbers were also found in other lymphoid organs including, unexpectedly, the thymus. It was also observed that at the time of peak immune response the thymus was strikingly acellular and generally contained less than 10 percent of the cell population considered normal for this organ.

NIH New Zealand albino rabbits, aged 4 to 5 months, were given a single intravenous injection of 5 μg of *S. enteritidis* somatic polysaccharide. At intervals ranging from a few hours to 51 days the animals were killed for immunologic study of their lymphoid tissues and histologic examination of the thymus. The thymus was excised and weighed; one lobe was fixed in formalin for histologic study, while the other was used for the enumeration of antibody-forming cells. Cell suspensions were prepared from the organ by gentle scraping against a 40-mesh stainless steel screen in Eagle's medium and the number of cells was determined by chamber counting. Cell morphology was examined in smears stained by Wright's method. In normal rabbits, aged 4 to 5 months, the thymus weighed approximately 4 to 5 g and generally contained 3 to 4 $\times$ 10^9 cells. Within 12 to 16 hours after administration of 5 μg of the somatic polysaccharide there was generally significant reduction in the weight of this organ and in its cell population. Five days after the injection of antigen the weight of the organ and its content of cells was most severely affected, the lowest values being 0.76 g and 3.2 $\times$ 10^7 cells, respectively. The localized hemolysis technique (*10*) was used to enumerate antibody-forming cells. Aliquots of 1 to 20 $\times$ 10^6 thymic cells were mixed with sheep erythrocytes that had been coated (*11*) with *S. enteritidis* polysaccharide, plated, incubated, exposed to guinea pig complement, and subsequently examined for zones of hemolysis. Controls consisted of parallel tests with normal sheep erythrocytes and occasionally of tests with cells coated with immunologically unrelated somatic polysaccharides. No evidence of antibody-forming cells in the thymus was obtained in 18 animals tested at intervals from 12 hours to 4 days and in 14 animals tested at intervals from 6 to 51 days after injection. However, 5 days after receiving 5 μg of somatic polysaccharide from *S. enteritidis* the thymuses of most rabbits (12 of a total of 13) contained significant numbers of cells secreting antibody specific for this polysaccharide (Table 1).

Five days after injection the thymus was grossly smaller, paler, and softer than normal. Microscopically there was striking acellularity, with loss of approximately 90 percent of the normal cell content; principally, loose, fatty, and fibrous tissue remained (Fig. 1). There was no real evidence of cellular destruction, nor was there an inflammatory response. Primarily the cortical cells were lost, with extremely few medium-sized or small lymphocytes remaining. A small amount of medullary element was left, composed of large reticular cells with pale cytoplasm and large pale vesicular nuclei. A very few small lymphocytes were scattered throughout. There was total absence of lobular differentiation and structural landmarks. The microscopic acellularity was much more marked than the gross diminution in organ size.

Some changes were discernible as early as 12 to 16 hours and possibly even sooner; these were slight loss of lobulation, some depletion of small and medium-sized cortical lymphocytes, and slight increase in the amount of loose, fatty, and fibrous tissue in the cortex. The loss of lobular differentiation, distortion of normal architecture, loss of cortical lymphocytic elements, and lastly the loss of medullary reticular

Table 1. Plaque-forming cells in rabbit thymus after injection of somatic polysaccharide.

Rabbit No. *	Number of plaques produced by 10^6 $\times$ No. of thymic cells							Average per 10^6 thymic cells†
	Plated on uncoated RBC			Plated on polysaccharide-coated RBC				
	20	10	5	20	10	5	1	
1		8			244	150		27
2		2		211	102	53	9	10
3			36		1640	1024		177
4			0			53	13	12
5		13		80	32			3
6	0			135	73		8	7

* Thymus from rabbits 5 days after intravenous injection of 5 μg of *S. enteritidis* somatic polysaccharide. † Values corrected for activity against uncoated RBC.

cells, were generally progressive with time, attaining a maximum at 5 days. An occasional specimen was not consistent with this progression.

Some increase in cellularity was detectable at 6 or 7 days; this increase was primarily lymphocytic and secondarily reticular. Very few prominent small lymphocytes appeared in the peripheral regions of the gland at this stage, and with time these increased in number. After these cells, medium-sized and some large lymphocytes appeared, reticular medullary cells increased, and lobular differentiation returned. Although mitotic activity was prominent at these stages, it was not greater than normal. At 22 days and later, the appearance was essentially that of a normal gland. Whether this return to normal appearance was the result of repopulation by cells from other sources or of thymic regeneration was not apparent.

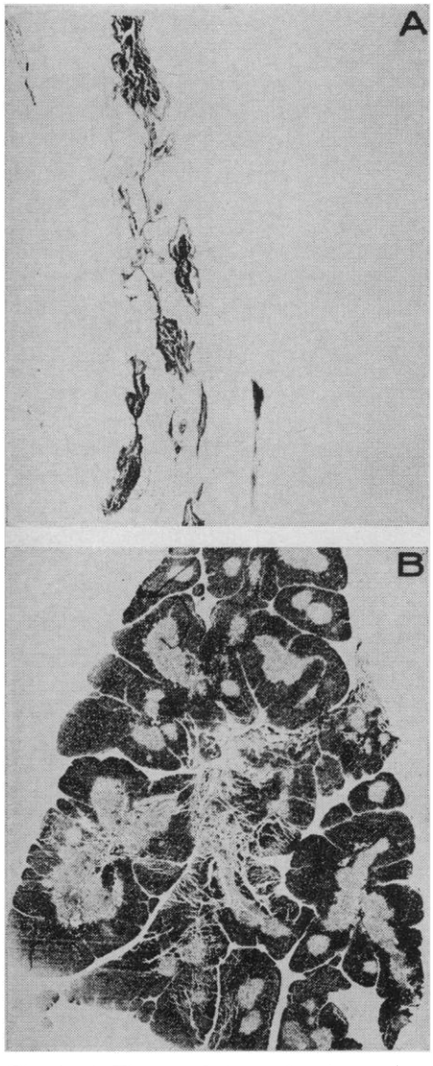

Fig. 1. *A*, Thymus 5 days after immunization, showing striking loss of cellularity. *B*, Thymus from untreated animal (hematoxylin and eosin, × 8.6).

Alterations in the mouse thymus in response to 100 μg of *S. typhi* endotoxin have been reported by Rowlands, Claman, and Kind (*12*). Gross decrease in size of this organ was most pronounced by the 3rd to 5th day after administration of endotoxin, and was associated with progressive destruction of lymphoid cells and thymic architecture; this was accompanied by a relative increase in the number of pyroninophilic cells.

In our study we have similarly seen consistent profound loss of cell population. However, in contrast with the findings in mice, no significant evidence of cell destruction was seen in the rabbit thymus. With respect to immune response to the endotoxic somatic antigens, the mouse generally fails to develop significant levels of agglutinins unless immunized intensively; the rabbit differs in promptly developing high titers of these antibodies in response to a relatively minute antigenic stimulus (*13*). This species difference and the methods employed may account for our finding of specific antibody-producing cells in the thymus of the rabbit. In addition to the differences in species reactivity, there are the further differences in the character of the endotoxin itself and the dose employed, any or all of which factors may decide the outcome.

Our most remarkable finding was the appearance of antibody-forming cells in the thymus of rabbits given a single systemic antigenic stimulus and their consistent association with a maximum loss of thymic cellularity. While morphologic changes in the thymus were evident soon after injection of antigen and became progressively more marked, it was only at the time of maximum histologic alteration and the lowest thymic-cell population that the antibody-producing cells were found in this organ. These findings suggest either that the thymus had been stimulated to produce antibody as a consequence of penetration of this organ by antigen, or, more likely, that this organ had been infiltrated by antibody-forming cells from other sites. It is to be noted that at 5 days the systemic response of the rabbit to 5 μg of this antigen includes production of relatively enormous numbers of plaque-forming cells in the spleen (average, 0.8 million) (*9*), and of lesser but substantial numbers of such cells in peripheral blood (*14*). It will be of value to ascertain whether these antibody-forming cells do in fact infiltrate the thymus.

There is considerable evidence that systemic administration of various antigens does not evoke the appearance of antibody-forming cells in thymus. To the best of our knowledge, only protein antigens were used in prior work; it may well be that our findings are attributable to unique properties of the particular category of antigens represented by the bacterial endotoxins. Whether other kinds of complex polysaccharides possess analogous antigenic capabilities or whether the release of corticosteroids and the diverse pharmacologic derangements elicited by this distinctive class of toxic antigens have in any way enhanced their specific antigenicity remains to be determined.

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References and Notes

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9. The designations endotoxin, somatic antigen, and somatic polysaccharide are used interchangeably and refer to the product extracted from gram-negative bacteria by the aqueous-ether procedure. These complex polysaccharides, containing minimum amounts of associated lipid and protein, display all antigenic and pharmacologic attributes characteristic of gram-negative bacteria. The preparation used in this work was provided by our NIAID colleague Edgar Ribi.
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