Lymphoepithelial Tissues of the Intestine and Differentiation of Antibody Production

Abstract. *The antibody response of young rabbits to the injection of killed* Brucella abortus *was abolished by prior extirpation of intestinal lymphoepithelial tissues combined with lethal x-irradiation and reconstitution with rabbit fetal liver cells. Extirpative surgery alone, like lethal x-irradiation and reconstitution without surgery, did not abolish this response. Intestinal lymphoepithelial tissues of rabbits apparently play a central lymphoid function and are responsible for the differentiation of the lymphoid cell line which produces specific antibody and immunoglobulins.*

The thymus plays a central role in the development of the lymphoid cell line responsible for cellular immunity. The importance of the thymus in the ontogeny of the lymphoid cell line responsible for specific antibody and immunoglobulin production is less well understood (*1*). Although neonatal thymectomy in rodents appears to interfere temporarily, at least, with the development of antibody responses to certain antigens, reports of normal amounts of circulating immunoglobulins and of specific antibody in children who fail to develop even the most primitive rudiment of the thymus place the role of the thymus in the development of humoral immunity in a different perspective (*2*).

Clinical, ontogenetic, and phylogenetic approaches to the study of immunity led us to postulate a two-component concept of the lymphoid system (*3*). We have reasoned that birds must not be unique in requiring a separate differentiative site for the development of an immunoglobulin- and antibody-producing system of lymphocytes and therefore we have sought tissue sites, an organ or organs, whose function in mammals and in man is homologous to that of the avian bursa of Fabricius. Our search has pointed to intestinal lymphoepithelial tissues as likely candidates in rabbits (*4*). The usefulness of this approach has been questioned (*5*), although the data presented in Miller's studies apparently indicate that the thymus influences the afferent side of the humoral response and are thus compatible with our hypothesis (*6*). We report that the differentiation of antibody-producing lymphoid cells involved in the efferent side of the humoral immune response is associated with intestinal lymphoepithelial tissues in rabbits.

Normal rabbits (4-week-old New Zealand White hybrids) were subjected to one of the following. Operation 1 consisted in excision of intestinal lymphoepithelial tissues (appendix, sacculus rotundus, and Peyer's patches); operation 2, thymectomy; operation 3: sham excision of intestinal lymphoepithelial tissues, splenectomy, and extirpation of mesenteric lymph nodes; operation 4, appendectomy (*7*). Other rabbits were unoperated controls. Lymphoid tissues in spleen and mesenteric lymph nodes approximate, in weight, the total amount of lymphoid tissue removed during operation 1.

All animals were bled at 8 weeks of age, and were then given intramuscularly 10^9 *Brucella abortus* killed organisms (*8*). Within 24 hours, the animals were lethally x-irradiated (1250 roentgens tissue dose) and immediately injected with 3 to 3.5 $\times$ 10^8 nucleated, viable cells from the liver of 20-day-old rabbit fetuses. Additional rabbits subjected to operation 1 were not x-irradiated. Antibiotics were supplied daily by intramuscular injections and in the drinking water. Rabbits were then bled 4, 7, 14, 21, 28, 35, and 42 days after immunization. *Brucella* agglutinin titers were measured by a micromethod modified from that of Wegmann and Smithies (*9*); the first dilution was 1:10. Our findings are summarized in Table 1.

The effect of x-irradiation upon antibody responsiveness of intact rabbits has been documented (*10*). Because of these earlier studies, we elected to expose our rabbits to the antigen for 24 hours before x-irradiation to avoid interfering with the afferent limb of the immune response. This decision appears justified in that a demonstrable response to the antigen could be seen as early as 4 days after x-irradiation. Rabbits whose intestinal lymphoepithelial tissues had not been excised showed early and significant antibody response.

Liver cells from 20-day-old rabbit fetuses were chosen for reconstitution of the lethally x-irradiated animals. This was done because outbred rabbits were used; at this stage of gestation, the thymus is just beginning lymphoid differentiation, and the hemopoietic stem cells injected can be considered minimally "contaminated" with immuno-

Table 1. Effect of lymphoid tissue extirpation and x-irradiation upon antibody responsiveness to *Brucella abortus* in rabbits. All surgical procedures were done in 4-week-old rabbits. Operation 1, excision of appendix, sacculus rotundus, and Peyer's patches; operation 2, thymectomy; operation 3, sham excision of appendix, sacculus rotundus, and Peyer's patches, excision of spleen and mesenteric lymph nodes; operation 4, appendectomy. All animals received 10^9 killed *Brucella abortus* organisms intramuscularly at 8 weeks and, where indicated, they received 1250 roentgens of total body x-irradiation 24 hours later; then they were reconstituted with liver cells from rabbit fetuses. *Brucella* agglutinin titers were measured by a micromethod, and the first dilution of serum was 1 : 10. Results are given as the mean ± standard error. The *P* value reflects Student *t*-test compared to response of unoperated x-irradiated rabbits. N.S., not significant.

Treatment		Log_2 titers																			
Operation	X-irradiation	4 days		7 days			14 days			21 days			28 days			35 days			42 days		
		No.	Mean	No.	Mean	*P*	No.	Mean	*P*	No.	Mean	*P*	No.	Mean	*P*	No.	Mean	*P*	No.	Mean	*P*
1	+	14		14	0.43 ±0.18	<0.01	9	0.11 ±0.13	<0.01	7	0.00	<0.01	6	0.00	<0.01	6	0.00	<0.01	5	0.00	<0.01
1	None	8	7.25 ±1.26	8	15.25 ±1.89	<0.01	8	15.25 ±2.03	<0.01	8	13.87 ±1.22	<0.01	7	9.86 ±1.28	<0.01	5	10.00 ±0.79	<0.01	5	8.60 ±1.04	0.05
2	+	18	8.75 ±0.75	18	10.00 ±0.88	<0.01	16	9.87 ±0.64	<0.01	15	10.20 ±0.97	<0.01	14	11.07 ±1.45	<0.01	11	8.82 ±0.88	<0.01	9	8.67 ±0.79	<0.05
3	+	15	3.53 ±0.25	15	5.53 ±0.38	N.S.	10	8.50 ±1.34	N.S.	5	9.60 ±2.54	N.S.	5	9.80 ±2.88	N.S.	3	9.00 ±2.55	N.S.	2	10.00 ±4.24	N.S.
4	+	11	0.91 ±0.26	11	2.00 ±0.49	0.001	10	5.50 ±1.07	N.S.	8	6.25 ±1.14	N.S.	5	7.40 ±1.30	N.S.	3	6.30 ±0.82	N.S.	2	6.00 ±2.83	N.S.
None	+	14		14	5.14 ±0.73		14	6.86 ±0.71		9	6.33 ±0.79		9	5.78 ±0.47		9	5.11 ±0.38		8	5.00 ±1.36	

competent cells. Hemopoietic tissue was successfully reconstituted in a significant number of animals. In all groups except those that received operation 1 and lethal x-irradiation, the animals that died after 8 days had *Brucella* agglutinin titers within the range achieved by the survivors of the respective group.

Extirpation of the appendix, sacculus rotundus, and Peyer's patches before x-irradiation and reconstitution, completely abolished the antibody response to *B. abortus* in 9 of 14 animals and markedly suppressed the response in the remaining five. When only the appendix, which represents 50 percent of this type of lymphoepithelial tissue of rabbits, was excised, the agglutinin response to *B. abortus* was delayed but not suppressed. By contrast, thymectomy or removal of the spleen and mesenteric lymph nodes did not interfere with the development of antibody responsiveness. We interpret these observations as reflecting the fact that although the thymus may play an important role in supplying antigen-sensitive cells during the afferent limb of the humoral immune response (*6*), it plays no role in the efferent limb of this response. Furthermore, removal of so-called peripheral lymphoid tissues (spleen and lymph nodes) does not interfere with recovery of humoral immunity after irradiation. Intestinal surgery of itself does not interfere with recovery; indeed, the response in the group of rabbits that received operation 3 was higher than that of the unoperated, irradiated control group. We believe that this finding probably reflects an adjuvant effect secondary to tissue injury.

In the absence of x-irradiation, removal of lymphoepithelial tissues of the intestine in 4-week-old rabbits is followed 4 weeks later by a humoral immune response to *Brucella* equal to that of normal nonirradiated rabbits. It would seem that by 4 weeks of age, rabbits have sufficient numbers of lymphoid plasma cell precursors that are differentiated but uncommitted and that retain the capacity to mount and participate in primary antibody responses 4 weeks later. Since the dose of x-irradiation probably was sufficient to destroy most of these cells we may conclude that we have prevented restitution of this cell line by operation 1. Under these circumstances, no primary antibody response can be mounted in spite of the antigenic potency of *Brucella*.

We reemphasize our postulate that stem cells originating in hemopoietic tissue and differentiating into immunoglobulin- and antibody-producing cells can develop and function normally without being directly or indirectly influenced by the thymus gland. Our results indicate that in rabbits, this differentiation occurs under the influence of lymphoepithelial tissues of the intestine. We believe that the data of Miller and associates (*6*) who used sheep erythrocytes as antigen in mice, support our general hypothesis that the thymus does not exert a differentiative influence upon antibody-producing cells. These investigators have clearly shown, however, that the thymus plays an important role in the development of antigen-sensitive cells operating on the afferent limb of the humoral immune response; this applies at least when the response to sheep erythrocytes is studied.

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Lunar Rivers

Abstract. *Mature meanders in lunar sinuous rills strongly suggests that the rills are features of surface erosion by water. Such erosion could occur under a pressurizing ice cover in the absence of a lunar atmosphere. Water, outgassed from the lunar interior and trapped beneath a layer of permafrost, could be released by a meteoritic impact and overflow the crater to form an ice-covered river. A sinuous rill could be eroded in about 100 years.*

Photographs obtained by the Lunar Orbiters show sinuous rills resembling meandrous channels of terrestrial streams; about 30 are visible from Earth and were first described in 1788 (*1*). The sinuous rills appear to originate in craters on relatively higher ground and to terminate on lower plains, their widths often decrease with distance from the crater, and they tend to occur in groups (2). Significant new features revealed by the Lunar Orbiter photographs are the smaller meandrous channel in the bottom of Rima Prinz I (Fig. 1) and the mature meanders in the smaller channel on the floor of Schroeter's Valley (Fig. 2), which require reexamination of theories of the origin of the rills.

The obvious similarities in appearance between the rills and terrestrial river channels early led to the suggestion that the rills were produced by erosion by water (*2, 3*). Since liquid cannot exist at pressures below the triple point, an atmosphere providing at least that pressure was considered essential if water was to flow on Moon's surface. The idea that aqueous erosion of a surface always requires an atmosphere has persisted hitherto, so that objections to the existence of such an atmosphere have also led to rejection of surface erosion by water as the mechanism forming sinuous rills (*4*).

Our main point is that the lunar surface may be eroded by water under vacuum conditions, since an overburden of ice can provide the pressure required to maintain the liquid phase. Thus we suggest that sinuous rills may indeed be features of water erosion of the surface by ice-covered rivers whose source is subsurface water released by meteoritic impacts.

Lunar subsurface water may be expected to result from outgassing from the interior. Because the subsurface