much of the ribulose diphosphate carboxylase of *T. neapolitanus* is contained within membrane-bound, polyhedral shaped inclusions. We suggest that structurally similar inclusions in other autotrophic microorganisms may also be "packages" containing this enzyme.

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Rabbit Blood Lymphocytes May Be T Cells with Surface Immunoglobulins

Abstract. *Rabbit peripheral blood lymphocytes and thymus cells do not respond to lipopolysaccharide mitogen in vitro, whereas spleen cells do. Soluble concanavalin A consistently stimulates 80 to 90 percent of rabbit peripheral blood lymphocytes, and the morphologic changes associated with such transformation may be observed within 18 hours after stimulation. Approximately 80 percent of rabbit peripheral blood lymphocytes have demonstrable immunoglobulin markers. These and other observations suggest that most rabbit peripheral blood lymphocytes are T cells with surface immunoglobulins.*

The observation that rabbit peripheral blood lymphocytes (PBL's) could be stimulated to undergo blast transformation with antiserum to immunoglobulin (anti-Ig) and antiserum to allotypic immunoglobulin (anti-As) provided the first convincing evidence that lymphocytes have surface immunoglobulins (*1*). The fact that up to 90 percent of rabbit PBL's could be transformed with anti-Ig indicates that most, if not all, rabbit PBL's have surface Ig (*2*). With the recognition of two cell types involved in the induction of immune responses, T (thymus-derived) cells and B (bone marrow–derived) cells (*3*), it became of interest to determine the classification of the rabbit PBL. Most observers concluded that the rabbit PBL is a B cell because of the association of surface immunoglobulin with mouse B cells and the inconsistent demonstration of surface Ig on mouse T cells [for example, see (*4*)]. Other methods of classifying T cells and B cells depend on their response to certain mitogens. Concanavalin A and phytohemagglutinin are believed to have mitogenic activity specific for T cells (*5*). In contrast, bacterial lipopolysaccharide or endotoxin (LPS) is believed to be a specific B cell mitogen (*5*). We now report that, on the basis of response to mitogens, the rabbit PBL behaves like a T cell.

Peripheral blood lymphocytes were obtained from rabbit blood that was defibrinated and sedimented with gelatin; the PBL's were cultured with different doses of mitogens (*1*, *6*). Four dilutions of each mitogen were tested on the cells of four or more individual rabbits; some of the results have already been presented (*6*). However, the most important mitogen, LPS, was not previously tested. Cultures of 3×10^6 PBL's in normal rabbit serum (18.5 percent) and Eagle's medium were set up, and doses of 5, 10, 50, and 100 μg of LPS (Bacto Lipopolysaccharide B, *Salmonella typhosa* 0901, control No. 553510, Difco) were separately added to individual cultures. Twenty-four hours later, 0.25 μc of thymidine was added, the incubation was continued for 24 hours, and the cultures were harvested. Part (1.5 ml) of each cell suspension was used for determining thymidine uptake and the remaining (0.5 ml) was smeared on a slide and stained with Jenner-Giemsa for the determination of the percentage of lymphocytes transformed. Similar experiments were performed with rabbit thymus (10×10^6 cells per culture) and spleen cells (3×10^6 to 5×10^6 cells per culture) (*6*) and graded doses of concanavalin A (*6*, *8*), pokeweed mitogen (Grand Island Biological, control A8218J), phytohemagglutinin, staphylococcal filtrate, anti-IgG (*2*), and anti-As (*1*, *6*).

Representative responses of spleen thymus and PBL's to the optimum doses of the various mitogens are given in Table 1. Cells from each source responded well to each of the above-mentioned agents. However, only spleen cells responded to LPS; PBL's and thymus cells give no responses even with doses up to five times greater and

Table 1. Optimum response of rabbit lymphoid cells to mitogens in vitro. Abbreviations: Con A, concanavalin A; PHA, phytohemagglutinin; LPS, lipopolysaccharide; PWM, pokeweed mitogen; Staph, staphylococcal filtrate; Anti-L, antiserum to rabbit light chain; Anti-γ, antiserum to rabbit IgG; and Anti-As, antiserum to rabbit allotypic determinants.

Agent	Spleen		Thymus		Blood	
	Blasts (%)	Radioactivity (count/10 min)	Blasts (%)	Radioactivity (count/10 min)	Blasts (%)	Radioactivity (count/10 min)
Control	20	4,123	5	5,000	<1	30
Con A	80	42,000	70	30,000	90	50,000
PHA	4+	22,000	2+	20,000	4+	2,000
LPS	50	17,000	6	4,500	1	50
PWM	60	15,000	35	20,000	30	1,200
Staph	60	14,000	30	10,000	60	4,600
Anti-L	50	35,000*	6	4,840*	90	6,000*
Anti-γ	45	34,000*	7	5,600*	80	5,000*
Anti-As	50	20,000	40	20,000	80	18,000

*Cultured in calf serum.

ten times less than the optimal mitogenic dose for spleen cells. The blast response to phytohemagglutinin is given as 1 to 4+ because agglutination and toxicity prevent a satisfactory evaluation of the percentages.

Further evidence for T cell properties of rabbit peripheral blood lymphocytes is that 80 to 90 percent of these cells consistently undergo blast transformation when stimulated with soluble concanavalin A, which is reported to stimulate mouse T cells specifically (*5, 9*); B cells may respond to soluble concanavalin A in the presence of a "factor" produced by T cells (*10*). However, 24 hours in vitro are required for the production of this factor, and the B cell response is measured after 2 to 3 days by thymidine incorporation only. The morphologic changes induced in rabbit peripheral blood lymphocytes by soluble concanavalin A may be recognized as early as 18 to 24 hours. Thus a B cell component in the response of rabbit PBL's to soluble concanavalin A appears unlikely.

Ultrastructural studies on PBL's and spleen cells labeled for surface immunoglobulin [by the mixed antiallotypic immunoferritin technique (*7*)] indicate that there is a marked difference in the structure of PBL's with surface Ig and the majority ($\sim$ 90 percent) of spleen lymphocytes with surface Ig. Approximately 80 percent of PBL's have surface Ig, do not contain endoplasmic reticulum, and have very little cytoplasm. In contrast, most of the labeled spleen cells have a higher density of surface label, significantly more cytoplasm, and at least some recognizable endoplasmic reticulum. Only 10 to 20 percent of the spleen lymphocytes are structurally similar to the PBL's.

Our observations indicate that, with respect to responses to mitogens, rabbit PBL's have properties associated with mouse T cells. Rabbit PBL's have the following definite advantages over other lymphoid cells for studies of blast transformation. (i) A relatively homogeneous population of lymphocytes is used. (ii) The transformation process occurs within 48 hours in vitro during which time cell death and cell division are negligible so that the percentage of blast cells identified at 48 hours accurately represents the number of cells in the original culture inoculum that is stimulated by a given mitogen (*1, 11*). (iii) The transformed cells may be identified morphologically as early as 18 hours after stimulation. (iv) Unstimulated cells remain small lymphocytes during the culture period. (v) Under optimum conditions up to 80 to 90 percent of the cultured cells may be transformed with anti-Ig or anti-As (*1, 2*) and a similar number have surface demonstrable Ig (*7*).

Thus, the finding that most rabbit PBL's respond to T cell mitogens but not to B cell mitogens, and have surface immunoglobulin implicates these cells as T cells with surface immunoglobulin. It is possible that LPS does not stimulate a B cell population unless T cells are present (*10*) and that in fact all the cells containing immunoglobulin are B cells. However, LPS stimulates cells from nude mice that have little or no T cell (helper) function (*9*), and LPS does not stimulate T cells (*5, 9*). It may be that two different B cells are required, that LPS stimulation requires B cell–B cell cooperation, and that only one B cell population is present in the peripheral blood. This possibility cannot be ruled out. Human peripheral blood lymphocytes also do not respond to LPS (*12*), but consist of different populations of cells with and without complement receptors (*13*). Those cells with complement receptors are believed to be analogous to mouse B cells (*14*) but may represent only one subpopulation of B cell. Approximately 20 percent of rabbit PBL's will bind sheep red blood cells coated with a complement (*15*). In addition, cells that have been transformed by mitogens, including antiserums to immunoglobulins, may have more complement receptors (*15*). However, this measurement is determined by combined autoradiography and rosetting and only a maximum of 5 percent of the cells are transformed by this measurement with any mitogen. Thus, the cells examined represent a minor contribution to the total transforming population.

The single finding of unresponsiveness to LPS does not necessarily prove the absence of B cells. However, the combined finding of unresponsiveness to LPS, uniform stimulation of 80 to 90 percent of the rabbit PBL's to soluble concanavalin A within 18 hours of stimulation, and the lack of complement receptors on 80 percent of the cells strongly implies that most rabbit PBL's are T cells. Even if 20 to 30 percent of the rabbit PBL's are B cells, the overlap in the population of cells responding as T cells to mitogens and cells containing surface immunoglobulin is so high (more than 50 percent of the total PBL's) that the conclusion that these cells are T cells with surface immunoglobulin must be seriously considered. At present there is conflicting evidence as to whether T cells do (*6, 16*) or do not (*17*) carry surface immunoglobulin. This point has, in fact, been the subject of controversy for an even longer time, as formulated by the question of the nature of the cell receptor functioning in the initial recognition of antigen by reactive cells and in the expression of delayed hypersensitivity reactions. The conclusion that rabbit PBL's are T cells on the basis of their response to mitogens must be tempered by the possibility that mitogens producing differential responses of mouse T and B cells may not do so for the rabbit.

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