Lymphocytopoiesis and Plasmacytopoiesis in Germfree Mice Stimulated With 7s Human Gamma Globulin[1]

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INTRODUCTION

Germfree animals have been described as having a reticulo-endothelial system (RES) of approximately one-half that of their conventional counterparts. This deficiency has been ascribed to smaller lymph nodes (1), fewer or complete absence of secondary nodules and fewer plasma cells (2), reduced serum gamma globulin levels (2), and less circulating antibody (3). Furthermore, it has been suggested that there is a longer latent phase in antibody production in the germfree animals (3). However, even with these deficiencies the germfree animal has been credited with the ability of making a satisfactory immune response to an antigenic stimulation.

To better understand this immune response it was decided to establish (a) the normal quantitative values for lymphocytopoiesis and plasmacyopoiesis in the nonstimulated germfree Swiss-Webster mouse, (b) to determine the quantitative changes in this cellular defense mechanism in response to a single antigenic stimulation of 7s human gamma globulin, (c) to determine the rate of proliferation of the lymphoid tissues in the nonstimulated and stimulated states using autoradiographic methods and tritiated thymidine (TH^3) as the isotopic label for DNA synthesis.

Material and Methods

To establish the differential counts and rate of proliferation of tissue in nonstimulated mice, germfree and conventional mice were injected I. P. with 1 μc TH^3/gm body weight and subsequently sacrificed at 2, 4, 24, and 48 hour time intervals. Samples of the mesenteric and cervical lymph nodes were removed, and teased to single cell suspensions in Earl's solution. The cell suspensions were brushed onto microscope slides, fixed overnight in 1% acid methyl alcohol, and coated with a nuclear emulsion, NTB-2, for autoradiography (4). After exposure times of 21 or 42 days the slides were developed for 2 minutes in Dektol, acid fixed for 4 minutes in Kodak Acid fixer, stained in Giemsa stain at 17° C for 5 minutes (5), and evaluated on the following basis: (1) Cells composing the differential count were classified as blasts, large, medium, and small lymphocytes (LL, ML, and SL), plasmoblasts (PB), immature plasma cells (IMPC), mature plasma cells (MPC), smears and macrophages (6). Counts consisted of 1000 cells. (2) The number of labelled cells of each type was recorded as was the total labelled cells per 1000 cells.

In antigen stimulated animals the antigen was injected I.P. 2 hours after TH^3. Animals were sacrificed starting at 1 day after

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TABLE 1
Quantitative Lymphocytopoiesis Values for Mesenteric and Cervical Lymph Nodes of Nonstimulated Germfree and Conventional Mice

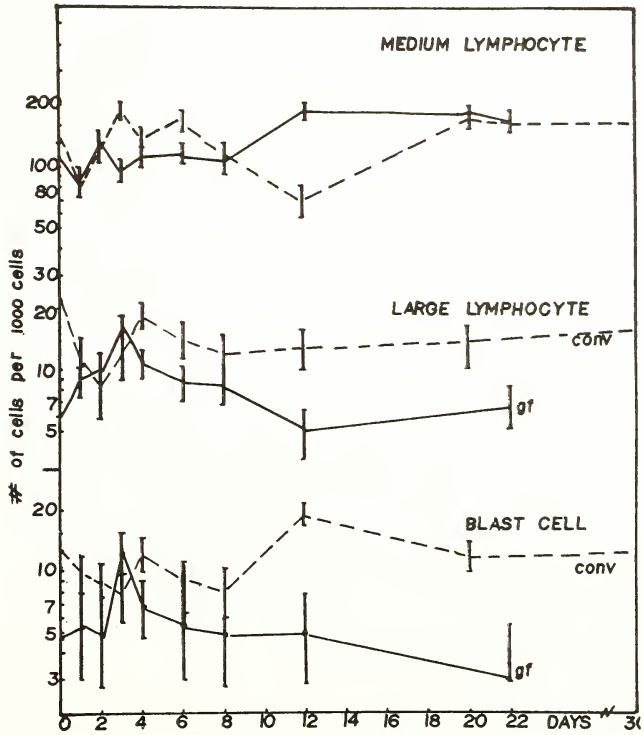
Type Node	Blasts	PB	LL	IMPC	ML	SL	MPC	Smears	Macrophages
Conv.									
Mes. (9)	13 ±2.8	2.9 ±0.4	23.5 ±4.9	6.8 ±1.1	134 ±11.7	708 ±13	1.3 ±0.6	97 ±8.2	12 ±2.9
Cerv. (9)	7 ±1.0	3.5 ±1.2	13 ±2.1	9.6 ±1.2	189 ±15.6	652 ±21	2.8 ±0.4	109 ±10.3	13 ±2.7
Germfree	4.9	0.75	6.1	1.5	114	742	0.5	124	7
Mes. (8)	±0.7	±0.35	±0.8	±0.3	±12	±20	±0.5	±14	±1.2
Cerv. (7)	3 ±0.25	1.3 ±0.3	4.4 ±0.75	4.6 ±0.65	173 ±14.9	670 ±33	1.1 ±1.2	139 ±21	2.4 ±0.6

and at designated times for the next 30 days. The lymph node suspensions were prepared as mentioned previously.

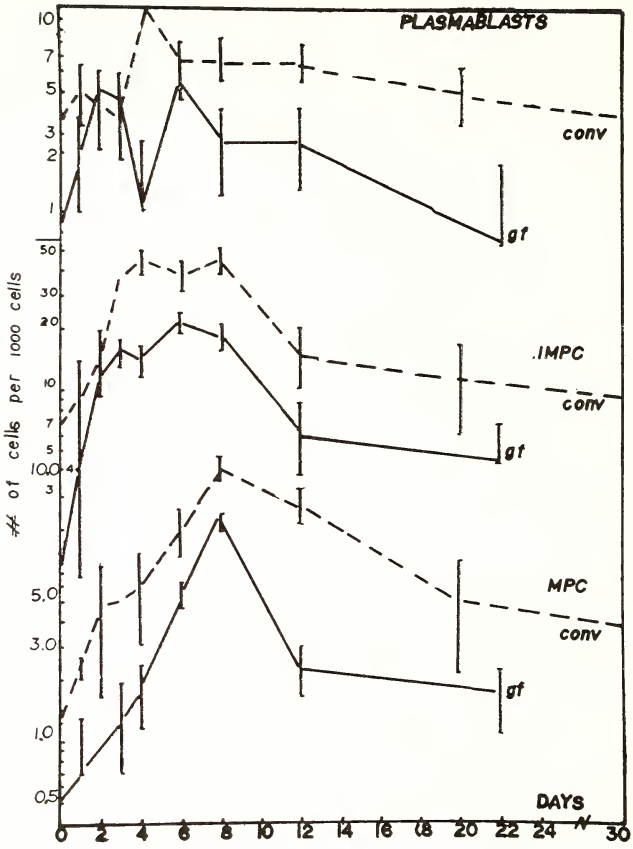
Results and Discussions

Table I shows the differential counts for lymphocytopoiesis of the mesenteric and cervical lymph nodes of nonstimulated germfree and conventional mice. If these cells are classified into two groups called (1) nonprimitive cells and (2) primitive cells based on their apparent ability to differentiate into cells capable of entering into the antibody response (blasts, PB, IMPC, and LL) (6), it is obvious that the germfree animal has one-third the amount of these primitive type cells as compared to the conventional mice. The quantity of these primitive cells in the germfree mouse does not differ between the mesenteric and cervical nodes whereas in the conventional animal the mesenteric nodes contain about 50% more primitive cells than does its cervical lymph node.

There is no significant difference between the number of ML and SL in these two groups, but there is a difference in the distribution of these two cell types in the nodes of these two groups. In both germfree and conventional conditions the cervical node contains more ML (about 18%) compared to 12.4% in the mesenteric node. There is



Graph I. Differential lymphocytopoiesis of mesenteric node of germfree and conventional mice stimulated with 7s human gamma globulin.



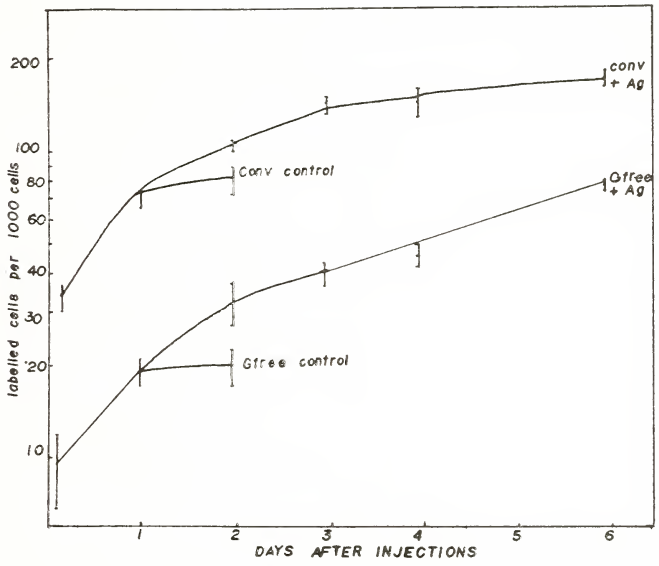
Graph II. Differential plasmacytopoiesis of mesenteric node of gremfree and conventional mice stimulated with 7s human gamma globulin.

approximately a factor 2 difference in the MPC distribution between the germfree and conventional mice. No difference was found in the number of recognizable macrophages between the cervical and mesenteric node of the conventional mice, but the germfree mice showed substantially less.

Following antigenic stimulation the statistical difference in the blasts, plasmablasts, and large lymphocytes of the mesenteric node in the germfree and conventional animals was lost for about an 8 day period (Graph I). This was due to a first day increase in these cell types in the germfree mouse while the conventional animals show a decrease during the first day. During this 8 day time interval the IMPC and MPC depict great proliferation with 7 and 17-fold increases respectively in conventional mice and 15 and 24-fold increases respectively for the germfree mice (Graph II). The peak quantity of these cells appear on the 3rd-4th day for the plasmablasts, the 4th-6th day for IMPC, and the 8th day post antigen injection for MPC. This shift

in cell types as a function of time depicts the maturation of the cells in plasmacytopoiesis. However, even at peak quantities for the IMPC and MPC the conventional mice have nearly two times the quantity of these cells as does the germfree animal. Thirty days after antigen stimulation the cellular components of the mesenteric node have returned to their normal base levels.

The proliferation of lymphoid tissue in the mesenteric node can be determined by the initial uptake of TH^3 in cells and the subsequent increase of TH^3 labelled cells due to cellular division. Graph III depicts three interesting items found in the mesenteric node of the germfree and conventional mice, when comparing the amount of labelled cells per 1000 cells found at various times after TH^3 injection and in the case of stimulated mice, an antigen injection. (1) In a given time period



Graph III. Cellular activity of mesenteric node of germfree and conventional mice stimulated with 7s human gamma globulin.

(2 hours) the conventional mice have a TH^3 uptake about four times greater than the germfree mice. (2) The rate of increase in the labelled cells as a function of time after TH^3 injection shows no difference in the rate of proliferation between these two groups. (3) In the antigen stimulated mice the increase of labelled cells was greater in both groups. A plateau was not reached after 1 day as was observed in the nonstimulated cases, but labelled cells continued to accumulate until about day 6.

One cannot interpret this 2 hour difference in uptake of label to mean a greater proliferation rate for the conventional mice because if this was the case the subsequent increase in labelled cells would be greater in the conventional mice and would be depicted by a line with a greater slope. The generation time of the cell population in nonstimu-

lated and stimulated germfree and conventional mice can be determined using the tritium index method (7), where

$$TH^3 \text{ index} = \frac{\text{number labelled } TH^3 \text{ labelled cells at X time}}{\text{number labelled } TH^3 \text{ labelled cells at 2 hours}}$$

A graphic plot of the index for a given set of data on the ordinate versus the respective time as the abscissus gives a convenient way of determining the amount of time needed for the index to be doubled. This time is considered to be an indication of the generation time (7). Table II shows that upon stimulation with a mild antigen this proliferation time is reduced from approximately 24 hours to 17 or 18 hours in both the germfree and conventional animal.

TABLE 2

Proliferation Time for Nonstimulated and Stimulated Germfree and Conventional Mice (Tritium Index Method)

	Nonstimulated	Stimulated
Germfree	26 hrs.	17 hrs.
Conventional	25 hrs.	18 hrs.

Walburg and Cudkowicy (8) by using whole organ scintillation detection, reported a longer proliferation time for lymphoid tissues in germfree animals. But these observations on the individual cell count suggest that what Walburg and Cudkowicy have reported is actually the difference in uptake of TH^3 label by the lymphoid tissues.

The following hypothesis consisting of two parts is advanced to explain this original difference in labelling. In the first place, the uptake of label is dependent upon cells undergoing DNA synthesis, and it has been reported that primitive type cells have a greater tendency for DNA synthesis than nonprimitive cells (6). Since the lymph node of the conventional animal has three times more primitive cells than its germfree counterpart it may be expected to take up more label in a given time period. Secondly, the germfree animal may have a physiological barrier in its node structure. Perhaps due to its protected environment and limited contact with antigenic stimulation, there is a restriction of blood flow to the lymph nodes, or a restriction of lymph circulation in the various peripheral and medullary sinuses. Consequently, the TH^3 label comes in contact with and is incorporated in the DNA of only those cells in the S phase that are adjacent to the unrestricted sinuses. These two factors would result in a greater uptake of label per unit of time in the conventional animal.

Summary

The quantative determination of lymphoid tissue shows the germfree animal to have about one-third the number of primitive type cells that may enter into an antibody response as compared to its conventional counterpart. Upon antigenic stimulation the amount of plasma cells in the germfree mouse remains approximately one-half that of the conventional animal although there is no difference in the time when these

cells reach their peak concentration. Auto-radiographic studies show there is no difference in the normal proliferation rate of lymphoid tissues of the germfree and conventional mice.

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