Antibody-Forming Cells in Five Organs of Hyperimmunized Guinea Pigs[1] (34924)

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There is little doubt at the present time that the spleen and lymph nodes play the major role in fostering antibody synthesis during the primary and secondary responses (1–7). Furthermore, estimates have been obtained that the spleen may have one-third of all the cells that are competent for antibody formation (3, 8). Therefore, the remaining two-thirds must be distributed in the other lymphoid organs (thumus, lymph nodes, and bone marrow) and possibly in other sites such as lungs, liver, etc., where the cells may be in transient passage.

If the progenitor cells are in an unstimulated small lymphocyte population (9, 10), they may be located anywhere in the animal's body since cells of this type are constantly in the thoracic duct (9) and are filtering through organs by way of the blood circulation (11).

Since plasma cells rarely circulate, any antibody-forming plasma cell found in a nonlymphoid organ must have journeyed there either as a blast cell or as small lymphocyte. Whether these cells lodged after being activated or were stimulated *in situ* is difficult to ascertain. We have shown by the hemolytic plaque procedure that a large number of antibody-forming cells can be detected in the lungs of C_3H mice during the secondary response (12).

The study reported here is an attempt to locate other organs that can collect such antibody-forming cells and attempt to estimate their number in relation to the spleen.

Materials and Methods. Preparation of purified soluble egg albumin–human gamma globulin (Ea-HuγG) conjugate was made by taking 80 mg of HuγG plus 20 mg of Ea dissolved in 9.0 ml of 0.1 *M* acetate buffer, pH 4; to this was added 1.0 ml of 5% glutaraldehyde. The preparation was allowed to react for 2 hr and was dialyzed first against water, then against 0.05 *M* borate buffer. Any resulting precipitation was removed by centrifugation and the solution was chromatographed on a 94 × 2.5-cm Sephadex G200 column. Elution was carried out with borate buffer (0.05 *M* borate and 0.05 *M* KCl). Fractions of 3 ml/tube were collected and analyzed by measuring absorbance at 280 mμ. The desired fractions were pooled and concentrated with polyvinyl-pyrrolidinone.

Immunization schedule. A group of guinea pigs was immunized with a preparation of Hu γG and Ea conjugate. Animals were sacrificed on days 15, 22, and 29. The initial injection into all animals was 0.5 ml of the 2 mg/ml antigen in Freund's adjuvant. Injections were made subcutaneously at two sites. The subsequent injections were given in 0.5-ml amounts intraperitoneally. The animals sacrificed on day 15 received two injections 1 week apart. The animals sacrificed on days 22 and 29 received a total of three injections 1 week apart.

Detection of precipitin and fluorescent antibody procedures. All animals at the time of sacrifice were bled and the serum was collected. The sera were tested for the presence of precipitin by the Ouchterlony agar method. At the time of sacrifice the spleen, liver, lungs, kidneys, and adrenal glands from each animal were mounted for study by the fluorescent antibody procedure.

The direct staining procedure was employed. Ea and HuγG were labeled with

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fluorescein. Serial tissue sections were alcohol-fixed, treated with either fluorescein-labeled Ea or HuγG for 1 hr and washed. The numbers of cells producing either Ea antibody or HuγG antibody were counted as follows. Tissue sections were observed with a Zeiss fluorescent microscope at a magnification of 250×; 300 individual fields were observed. The number of antibody-forming cells in each field was tallied and the results were reported as number of antibody-forming cells/100 fields.

Results. Spleen sections treated with fluorescein-labeled Ea showed staining of plasma cells. Similar sections treated with fluorescein-labeled rabbit anti-Ea or rabbit anti-HuγG were negative. The direct staining of the cells with fluorescein-labeled Ea could be inhibited by pretreating the section with 20 mg/ml of solution of Ea, but not by pretreatment with HuγG. Similar studies were made to establish the specificity of staining by fluorescein-labeled HuγG.

The possibility that the cellular staining may be due to antibodies adsorbed on the cells was tested by removing 4 ml of blood from normal guinea pigs and replacing this blood by injecting 4 ml of guinea pig serum containing 2 mg/ml of antibody protein to Ea and 2 mg/ml of antibody protein to HuγG. The sera from these animals tested at 24 hr contained antibodies to the respective antigens. Tissue sections tested by the direct fluorescence staining method for Ea or HuγG antibody-containing cells were negative. Based on these results, the cellular staining observed in the various organs of immunized animals presumably is due to cells synthesizing antibodies rather than cells that have adsorbed antibodies on their cytoplasm.

To gain a relative distribution of antibody-forming cells in the various tissues, the number of antibody-forming cells detected/100 fields for each tissue was totaled. For example (Table I) in animal No. 286 a total of 468 Ea antibody-producing cells and 1428 HuγG antibody-producing cells were counted. 452 Ea antibody-forming cells or 96.6% of there were found in the spleen. Similarly 1348 of the HuγG antibody-forming cells or 94.4% were found in the same organ. Table I summarizes the responses in animals injected with a soluble preparation of conjugated Ea–HuγG. In all cases the response to HuγG was predominant and prolonged. The results show a fair number of antibody-forming cells in the lungs. Approximately 70–96% of the total number of antibody-forming cells were found in the spleen, 1–16% in the lungs and 0–14% in the liver per 100 fields surveyed.

Of the organs studied, the lungs consistently showed the presence of antibody-containing cells. The liver was less consistent in showing the presence of these cells. The kidneys and adrenal glands were variable but in most cases the adrenal glands were negative. In animal No. 285, an exceptionally large number of antibody cells was found in the kidneys (3.5%).

The use of two antigens with the fluorescent antibody method permits the evaluation of two antibody cell types in the various organs of a single animal and serves as a double check on the distribution of antibody cells in the organs provided the antibody responses to both antigens are satisfactory. This internal consistency was clearly seen for animals Nos. 286–288 and 278 when the percentages of antibody-containing cells found in the various organs against Ea and HuγG were calculated (Table I). There were variations among individual animals as seen with animal No. 279. The presence of Ea or HuγG antibody-containing cells in the organs appeared to depend on the total number present in the spleen. In general, the greater the number of antibody-containing cells found in the spleen the greater was the number distributed to other organs. The percentage of antibody-forming cells in the various organs plus or minus the standard deviation of the mean based on the total response of the 29-day group was as follows: spleen 81.18 $\pm$ 5.00, lung 12.26 $\pm$ 3.91, liver 5.48 $\pm$ 2.05, kidney 0.40 $\pm$ 0.18 and adrenal gland 0.64 $\pm$ 0.37.

Histologically large confluent areas of plasma cells containing antibodies were seen in the red pulp regions of the spleens of highly stimulated animals. A few antibody-containing cells in mitosis were also seen. In the other organs the distribution of cells ap-

TABLE I. The Response of Guinea Pigs Injected with Glutaraldehyde-Treated Egg Albumin and Human Gamma Globulin (soluble antigen conjugate).[a]

Guinea pig no.	Days[b]	Precipitin		No. of antibody cells/100 fields									
				Spleen		Lung		Liver		Kidney		Adrenal	
		Ea	Huγ	Ea	Huγ	Ea	Huγ	Ea	Huγ	Ea	Huγ	Ea	Huγ
282	15	+	+	17	308 (85.3)	—	20 (5.6)	1	33 (9.1)	—	—	—	—
283	15	+	+	5	32 (55.2)	—	26 (44.8)[c]	—	—	—	—	—	—
284	22	—	—	9	16	—	—	—	—	—	—	—	—
285	22	+	+	254 (85.8)	1505 (87.2)	38 (12.8)	151 (8.7)	—	9 (0.5)	4 (1.4)	61 (3.5)	—	—
286	29	+	+	452 (96.6)	1348 (94.4)	5 (1.1)	39 (2.7)	8 (1.7)	37 (2.6)	3 (0.6)	—	—	4 (0.3)
287	29	+	+	256 (71.3)	609 (70.4)	52 (14.5)	137 (15.8)	50 (13.9)	116 (13.4)	1 (0.3)	3 (0.3)	—	—
288	29	+	+	54 (93.1)	604 (86.2)	4 (6.9)	70 (10.0)	—	24 (3.4)	—	3 (0.4)	—	—
278	29	+	+	107 (87.7)	268 (84.0)	6 (4.9)	31 (9.7)	6 (4.9)	13 (4.1)	3 (2.5)	2 (0.6)	— —	5 (1.6)
279	29	+	+	14	56 (82.4)	18	8 (11.8)	—	4 (5.9)	—	—	2	—

[a] % Antibody-forming cells found in the organs given in parentheses for those animals when a total of 50 or more antibody-forming cells were detected/100 fields.

[b] Day sacrificed.

[c] This value appears to be high.

peared to be somewhat random. In the lungs the plasma cells could be seen in the stroma surrounding pulmonary vessels and also in the alveolar walls; occasionally cells could be found within the alveoli. In the liver, plasma cells containing antibody were seen in the sinusoids and the stroma around blood vessels. In the kidney, cells could be found only occasionally; but when seen, they were near glomerular tufts and in the connective tissue between the tubular epithelium. The liver, kidneys, and adrenal glands showed no large confluent masses of plasma cells, whereas in the lungs many cells could be observed which were in greater aggregation than in the other organs.

Discussion. In previous studies when mixtures of Ea and HuγG were given to guinea pigs, there was a sequential response to the antigens. The animals responded first to HuγG, then to Ea. In spite of large numbers of cells producing antibody to HuγG, precipitating antibody was not detected in the serum initially. Even after a second stimulation the HuγG cellular response continued to decline, whereas the cellular response to Ea accelerated (13).

To obtain a more simultaneous response, Ea and HuγG were treated with glutaraldehyde in an attempt to polymerize the antigens (14). A simultaneous response was desired to determine the extent of localization of two types of antibody-forming cells in the various organs. Preliminary studies showed the glutaraldehyde-treated antigen preparations, in contrast to Ea and HuγG as antigen mixtures, gave a very limited response even when adjuvant was employed.

It has been reported that shortly after antigen treatment there is a loss of small lymphocytes from the spleen (4, 7, 15, 16). Some of these cells are in active synthesis while others undoubtedly are carrying information to synthesize more antibody. The possibility that these cells can seed the extralymphoidal organs cannot be neglected.

These studies indicate that the lungs and liver appear to be prime sites for localization of antibody-forming cells. We believe that such cells arise primarily from the lymphoid organs where the initial events of antibody synthesis begin. Presumably, migratory precursor cells pass by way of the circulation (11, 17) and seed the various organs and under secondary stimulation actively divide and synthesize antibodies. Evidence for this mechanism was obtained by studying the appearance of plaque-forming cells in the lungs of C_3H mice (12).

Although it has not been possible to quantitatively estimate the total number of antibody-containing cells in the lungs or liver contributing to the total antibody synthesis, it appears that both organs contain a significant number of cells. Of the organs studied in the hyperimmune animals approximately 81, 12, and 5% of the antibody-forming cells were in the spleen, lung, and liver, respectively. In the normal guinea pig the mass of the lungs is 5 times greater, and the mass of the liver 30 times greater than the spleen. If these considerations are taken into account, the total contribution of antibody cells by these organs may equal or even exceed the contribution by the spleen. The secondary role played by these major organs in capturing antibody-forming cells and permitting antibody synthesis to proceed requires further study. Whether progenitor cells reside in these organs at all times and become activated *in situ,* or whether they locate there by mechanical filtration from the circulation after being activated in the lymphoid complex has not been determined.

Summary. The simultaneous cellular antibody response to two antigens was studied in guinea pigs made hyperimmune to a glutaraldehyde-treated antigen mixture of egg albumin and human gamma globulin. In such animals varying numbers of antibody-forming cells could be detected in the lungs, liver, kidneys, and adrenal glands. The lungs and liver appeared to be major sites of antibody synthesis. If these cells are the progeny of potential antibody-forming cells stimulated *in situ* or if these cells were stimulated in lymphoid organs and filtered from the circulation is not known.

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