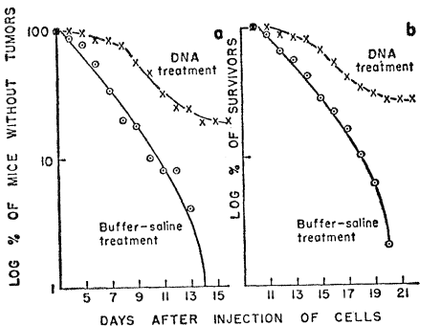

Fig. 1. (*a*) The delayed appearance of tumor nodules, and (*b*) the prolongation of life in mice given L1210 cells treated with thymus DNA.

abolished their inhibitory effect on tumor growth. The temperature denaturation curve for our DNA preparations was sharp and had a temperature midpoint at 86°C, as expected for native thymus DNA (*7*). Moreover, during the DNA isolation procedure, the DNA was extracted repeatedly in a chloroform-butanol mixture to remove contaminating protein, and ribonuclease was sometimes used to destroy any traces of RNA in the DNA preparation.

It is most unusual that a natural molecule such as thymus DNA could prevent the growth of the tumors in as many as 19 percent of the experimental animals. Even in those animals that did develop tumors after injection of DNA-treated cells, the DNA must have affected the vast majority of the cells for the following reasons. On the basis of two cell-generations per day for L1210 leukemia cells, tumors became palpable in about 14 cell-generations (7 days) in control animals and in about 20 cell-generations (10 days) in the experimental animals (Table 1). Let us assume that, when first detected, palpable tumors are composed of a relatively constant cell number. The number of cells which must have been altered by the DNA treatment can now be calculated. If $2^{14}N = 2^{20}(N - n)$, then $n = 98$ percent of N, where N is the total number of viable cells injected per animal, and n is the number of DNA-treated cells which do not form tumor.

Surprisingly, 98 percent of the cells are susceptible to the action of DNA. The average lag of 3 days in the appearance of tumors of DNA-treated cells in comparison with control cells can be accounted for by the survival of only 2 percent of the leukemia cells following DNA treatment. It is, of course, possible that DNA affected all the cells by temporarily delaying or permanently lengthening, the cell-generation time, but this would not readily explain how tumors were sometimes completely suppressed. More likely, any animals that survived free of tumors did so because most of the DNA-treated cells were no longer able to form tumors, and because the animals may have been injected with none or too few of the unaltered cells.

Apparently, thymus DNA did not merely destroy the leukemia cells during the incubation period, because cell viability, as determined by the eosin procedure, was unaffected by DNA. Eaton *et al.* (*4*) had previously demonstrated, by means of metabolic inhibitors and cell-dilution and cell-respiration techniques, that only nonviable ascites tumor cells were stained by eosin. In our experiments, only about 25 percent of the cells that had been incubated, with or without DNA, were permeable to eosin. Yet, the DNA-treated cells may have survived the incubation period only to succumb in the animal.

We strongly doubt that chromosomal genetic transformation would explain our results, since Szybalska and Szybalski (*2*) found that transformation for a specific genetic marker was less than 0.1 percent of the total number of mammalian cells exposed to DNA. However, the exogenous DNA may have acted either independently or together with some endogenous factor(s) in the cytoplasm or in the extrachromosomal portion of the nucleus in order to prevent the cells from forming tumor nodules.

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References and Notes

1. F. M. Sirotnak and D. J. Hutchison, *Biochim. Biophys. Acta* **36**, 246 (1959); S. M. Gartler, *Biochem. Biophys. Res. Commun.* **3**, 127 (1960); M. R. Chorazy, H. H. Baldwin, R. K. Boutwell, *Federation Proc.* **19**, 307 (1960); E. Borenfreund and A. Bendich, *J. Biophys. Biochem. Cytol.* **9**, 81 (1961); E. R. M. Kay, *Nature* **191**, 387 (1961); T. Wilczok, *Neoplasma* **9**, 369 (1962).
2. E. H. Szybalska and W. Szybalski, *Proc. Natl. Acad. Sci. U.S.* **48**, 2026 (1962).
3. T. R. Bradley, R. A. Roosa, L. W. Law, *J. Cellular Comp. Physiol.* **60**, 127 (1962).
4. M. D. Eaton, A. R. Scala, M. Jewell, *Cancer Res.* **19**, 945 (1959).
5. R. D. Hotchkiss, in *Methods in Enzymology*, S. P. Colowick and N. O. Kaplan, Eds. (Academic Press, New York, 1957), vol. 3, p. 708.
6. G. F. Gitlitz, A. G. Ship, J. L. Glick, A. H. Glick, *J. Surgical Res.* **3**, 370 (1963).
7. P. Doty, J. Marmur, N. Sueoka, *Brookhaven Symp. Biol.* **12**, 1 (1959).
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Focal Antibody Production by Transferred Spleen Cells in Irradiated Mice

Abstract. *Lethally irradiated mice were injected with small numbers of normal spleen cells and then immunized with sheep erythrocytes. Antibody activity was found in their spleens in localized areas whose number corresponded to the number of spleen cells injected. When sheep and pig erythrocytes were injected together, antibody against each was found in separate areas. Each area may consist of the progeny of a single precursor cell, restricted to forming a single antibody.*

Our understanding of lymphoid-cell differentiation would be advanced if the antibody-forming descendants of a single cell could be tested for their response to several antigens. In the absence of an ideal technique for studying antibody-forming cells in vitro, something may still be learned from their behavior under controlled conditions in vivo. We now report experiments in which normal (nonimmunized) lymphoid cells were injected into lethally irradiated syngeneic mice and then induced to make a primary response to foreign erythrocytes (*1*). By using small numbers of donor cells and studying the distribution of specific hemolysin in the recipient spleens, we have shown that the earliest production of antibody is localized in a few small highly active areas, whose number depends on the number of donor cells injected. These areas were found in the central white pulp of the spleen —that is, the normally lymphopoietic part—and not in the hemopoietic nodules (*2*), which do not contain lymphocytes or antibody (*3*). Lymphoid nodules of the type derived from phytohemagglutinin-stimulated donor cells (*4*) were not seen in our experiments.

Mice, LAF_1, aged 12 to 16 weeks were given a single exposure of either 900 roentgens of 250-kv-peak x-rays or 1260 r of Co^{60} γ-rays; this exposure was followed by an intravenous injection of spleen cells from a normal syngeneic mouse. These cells were prepared by cutting the spleen in pieces and teasing out the contents, which were then made into a single-cell suspension by gentle aspiration through a series of needles. On the 1st and 4th days after irradiation, 2×10^8 washed sheep erythrocytes were injected intravenously. On the 8th day the mice were killed, and their spleens were dissected under the stereomicroscope. The spleens were first cut transversely into 12 numbered slices, each about 1 mm wide, and then from each slice the white pulp was isolated, by trimming off the red pulp, and cut into about 10 pieces. Whenever possible the nodular shape of the white pulp pieces was preserved; however, since the white pulp is a continuous tissue sheathing the arterial tree, discrete nodules or colonies could not be obtained. The pieces from each slice were placed on a 1.4-percent agar layer in a petri dish and overlaid with 0.7-percent agar containing sheep erythrocytes, essentially as in the single-cell hemolysin assay (*5*). After an hour at 37°C and the addition of complement, zones of hemolysis appeared around some of the pieces, indicating that antibody had diffused from them. When the number of active pieces was small, they were invariably localized to one or two adjacent slices; in a few spleens, sliced and numbered in three dimensions, the activity could often be localized to a roughly spherical region, comprising about 2×10^5 to 2×10^6 cells. When a slice, or slices, containing one or more active pieces was flanked by slices without activity, it was designated as an active area. Because the original position of each slice in the spleen was known, we were able to construct a map of antibody activity for each spleen, and to estimate the number of active areas.

Mice that were irradiated and immunized, but given no spleen cells, served as controls. Bone marrow was not given, since prolonged survival was not required, and all the mice appeared healthy at autopsy.

In 30 spleens from mice receiving 1×10^6 spleen cells, an average of about two active areas was found, with a variation closely fitting the Poisson distribution (Table 1). No particular region of the spleen seemed to be significantly favored as the site of activity. In another 41 mice receiving different numbers of spleen cells, the number of active areas was in proportion to the number of cells injected (Fig. 1). Not more than three active areas in one spleen could be separately distinguished with accuracy, on account of confluence, so that the results for cell doses above 1×10^6 are not as reliable as those for the lower doses, where most of the spleens had either one area or none. In ten mice receiving 2×10^5 or fewer cells, no activity was found. Varying the dose and timing of antigen, the day of assay, the number of slices cut, the extent of trimming and the size of white pulp pieces, or the time of incubation and concentration of complement, influenced somewhat the degree of lysis, but these factors did not alter the average number of active areas found. The degree of lysis also varied considerably within each experiment, and often within each spleen; however, the distinction between active and inactive areas was always clear-cut. Therefore it seems likely that each active area originates from some element in the donor inoculum, quite possibly from a single precursor cell.

If this is so, a method is available which makes it possible to single out the precursors of antibody-forming cells, at least on a functional basis, and study the immunological responses of their progeny. Our results (two active areas per 1×10^6 spleen cells injected) refer to all areas with detectable activity; about half of these contained pieces with significantly more activity, as judged arbitrarily by a zone of lysis at least 1 mm in radius. These areas were designated "++" and are listed separately in Tables 1 and 2 and Fig. 1. Whether the distinction depends solely on the numbers of antibody-forming cells in each piece, or whether there are qualitative differences in the antibodies, remains to be established. Great heterogeneity is found among the antibodies formed against even a simple determinant (*6*).

The number of antibody-forming cells per active area was not measured directly, but spleen pieces of similar size from a nonirradiated mouse, immunized with sheep cells, produced "++" lysis by the 4th day. At this time eight pieces, instead of being plated, were tested for their content of single plaque-forming cells, and contained 200 ± 117. This, then, may be a rough indication of the number of plaque-forming cells responsible for an active piece in the irradiated animals; an active area might contain from one to five such active pieces, and therefore, at the most, about 1000 plaque-forming cells, which would represent ten divisions of a single cell.

The figure of two areas, and therefore two postulated precursor

Table 1. Distribution of antibody against sheep erythrocytes in 30 spleens from mice receiving 1×10^6 normal spleen cells.

Active areas (No.)	Spleens (No.)	Expected from Poisson distribution (No.)	χ^2
All active areas (mean>1.96 per spleen)			
0	3	4.05	0.56
1	6	8.13	0.69
2	10	8.13	0.53
3 or more	11	9.69	0.21
	(P=0.3 for 2 degrees of freedom)		
"++" active areas only (mean>1.1 per spleen)			
0	9	9.99	0.10
1	10	10.99	0.09
2	9	6.04	1.45
3 or more	2	2.98	0.48
	(P=0.3 for 2 degrees of freedom)		

Table 2. Calculation of the fraction of the injected precursor cells that give rise to active areas in the spleen. For the first irradiated hosts the numbers show the predicted numbers of active areas per spleen; these are equivalent to the number of precursor cells in the spleen. For the second irradiated hosts, injected with half a spleen from the first hosts, the number of active areas expected would be equal to the number of precursor cells injected.

Active areas	First host (No.)	Second host	
		Expected (No.)	Determined (No.)
	Expt. 1: 50 × 10⁶ cells		
All	20	10	0.25
++	11	5.5	0
	Exp. 2: 50 × 10⁶ cells		
All	100	50	1.75
++	55	27.5	1.0

Table 3. Distribution of antibody against sheep and pig erythrocytes (rbc) in 20 spleens from mice receiving various numbers of normal spleen cells. In each vertical column, every number represents a single mouse; the numbers in columns 2, 3, and 4 represent the same animals.

Cells injected (No.)	Number of areas active against rbc of:		
	Sheep	Pig	Sheep+pig
4×10^5	0,1,1	1,1,2	0,0,0
	1,0,1	1,1,0	0,0,0
1×10^6	1,0,2,0	1,1,1,1	0,0,0,0
	1,2,2,0	2,2,2,1	0,1,2,0
1.5×10^6	3,2,1	0,2,2	0,1,1
	2,3,3	1,2,2	0,1,1

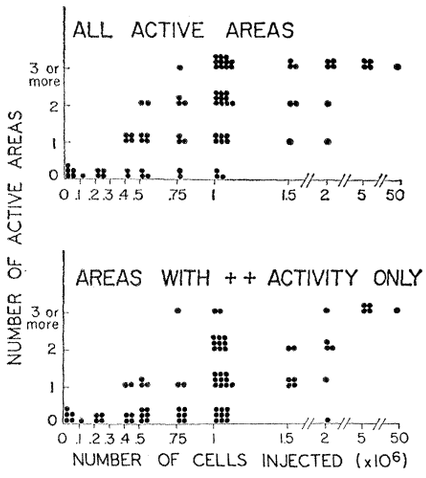

Fig. 1. Distribution of antibody against sheep erythrocytes in 81 spleens from mice receiving various numbers of normal spleen cells.

cells, per million injected spleen cells, is clearly a lower limit, since not all the injected cells can be expected to settle in the spleen. The fraction which does can be estimated by reinjecting the whole or a part of a spleen whose content of precursor cells is already known. For example, in one experiment 50×10^6 normal spleen cells were injected into four irradiated mice, and 1 day later their spleens were pooled and reinjected into another eight irradiated mice, which were then immunized and assayed as usual. As Table 2 shows, a considerable reduction in activity resulted from the transfer, and therefore presumably from any such transfer, so that only about 4 percent of the injected precursor cells settle in the spleen. Thus the two active areas of antibody against sheep erythrocytes normally derived from a million spleen cells represent about 50 precursor cells in the inoculum, which is equivalent to 1 per 20,000 cells, or about 5000 per normal spleen.

A major question at this stage is whether the active areas discussed above contain all the antibody activity in the irradiated spleen, or only the activity against sheep erythrocytes. To investigate this, the response to a second, noncross-reacting antigen was simultaneously measured. The mice were immunized with 2×10^8 pig erythrocytes at the same time as with the sheep erythrocytes. For the assay a second agar overlay, containing pig erythrocytes, was poured on top of the first, which contained the sheep cells (or vice versa). The layer in which lysis occurred could be identified by focusing up and down with the stereomicroscope, or by peeling off the top layer to reveal the lower. In nonirradiated mice injected with sheep cells or pig cells or both and assayed on double-overlay plates, lysis consistently developed in the appropriate layer or layers, regardless of which layer was on top; no cross-reaction was detectable under these conditions. In the nonirradiated, doubly immunized animals, killed on the 4th day after immunization, essentially every piece of the spleen white pulp (300 to 400 pieces) produced lysis of both types of erythrocyte. In 20 irradiated animals given normal spleen cells and then doubly immunized, activity against both sheep and pig erythrocytes was detectable, but in different areas (Table 3). With the larger spleen-cell doses there was occasional overlapping, but maximum activity against both antigens was never found in the same piece. Five spleens had active areas against pig cells only; two had activity only against sheep cells.

We conclude that if single precursor cells are in fact responsible for the areas of localized antibody formation, which are apparently of a single specificity, the progeny of each precursor must be restricted to forming exclusively, or predominantly, a single type of antibody. Whether this restriction is imposed by contact with the antigen, or whether it is inherent in the precursor cell itself, remains to be demonstrated.

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References and Notes

1. T. Makinodan, E. H. Perkins, I. C. Shekarchi, N. Gengozian, in *Mechanisms of Antibody Formation*, M. Holub and L. Jaroškvá, Eds. (Academic Press, New York, 1959), p. 182.
2. J. E. Till and E. A. McCulloch, *Radiation Res.* **14**, 213 (1961).
3. T. Mekori and M. Feldman, *Transplantation* **3**, 98 (1965).
4. T. Mekori, L. Chieco-Bianchi, M. Feldman, *Nature* **206**, 367 (1965).
5. N. K. Jerne and A. A. Nordin, *Science* **140**, 405 (1963).
6. H. N. Eisen and G. W. Siskind, *Biochemistry* **3**, 996 (1964).
7. Supported by special grants E-38 and E-332 of the American Cancer Society, California Division, and PHS grant AI-06501-01 to B.W.P.

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Interocular Transfer in Goldfish: Color Easier than Pattern

Abstract. *Goldfish were trained to monocularly discriminate different patterns that also differed in color. When tested with reversed combinations of color and pattern cues, the fish responded on the basis of pattern while using the trained eye but on the basis of color while using the untrained eye. Interhemispheric transfer of color information was therefore more effective than that of pattern.*

Binocular integration of visual behavior has special application among those vertebrates whose optic pathways are completely crossed. In the fish, visual input is projected from each eye only to the contralateral optic tectum (*1*). However, visual information must reach the ipsilateral half of the brain, because fish trained to discriminate colors or shapes monocularly can discriminate effectively when tested while using the untrained eye alone (*2*). The phenomenon of interocular transfer implies that each half of the brain has access to visual information that reaches it by way of two separate channels: (i) the direct retinotectal input and (ii) information arriving by way of certain unspecified commissural pathways. The function of these commissures is analogous to that of the corpus callosum of mammals, which relays visual information between the cerebral hemispheres of cat and monkey (*3*).

The intact fish apparently behaves much like a mammal with a split optic chiasma, in which visual input travels to only one-half of the brain. Like mammals prepared in this way (*3*), goldfish show definite limits to interocular transfer (*4*) and also can learn to discriminate opposing shapes by way of opposite eyes (*5*). Neither in fish nor in mammals do the commissural pathways transmit an identical copy of the visual world as viewed by the directly stimulated half of the brain. For these reasons, new paradigms developed for analysis of interocular equivalence in fish should prove useful in exploring the functions of the more complex mammalian brain.

Most investigations of interhemispheric relations in various mammals have been concerned with measurement of interocular transfer after various midline surgical transections. The extent of transfer varies with the meth-