INDUCTION OF ANAMNESTIC RESPONSE TO BSA BY TRANSFER OF "PRIMED" LIVER CELLS*

Donna L. Vredevoe and Eric L. Nelson

Department of Bacteriology, University of California, Los Angeles and Allergan Pharmaceuticals, Santa Ana, California.

Received November 8, 1962

The liver is believed incapable of synthesizing gamma globulin (Askonas, Humphrey and Porter, 1956 and Miller and Bale, 1954), nevertheless Kupffer cells take up substantial amounts of injected antigens (Halpern, 1959). Garvey and Campbell (1956, 1957) reported localization and persistence of S^{35} labeled antigens in rabbit livers. The labeled proteins were found associated with a salt soluble nucleic acid containing liver fraction which was 100 to 200 fold more antigenic than the original antigen.

Rittenberg and Nelson (1960) proposed that digestion of antigen within macrophages was a necessary step leading to production of antigenic information which likely was contained in nucleoprotein capable of directing antibody production. The antigenic information from macrophages induced antibody in cells capable of globulin synthesis. Rittenberg and Nelson suggested that the macrophagerich liver would be a logical organ in which to study induction of information for stimulating antibody formation in other tissues.

We believed that it might be possible for a nucleoprotein messenger or other mediator of information for antibody formation, pre-

*This investigation was supported in part by a Public Health Service fellowship (number GF 14,520) from the Division of General Medical Sciences, USPHS Health Service, a UCLA Graduate Fellowship in Microbiology, contract No. NONR-233-(68) with the office of Naval Research

sumably released from cells phagocytizing antigen, to be transmitted across a millipore filter membrane to cells capable of antibody production. We therefore determined the antibody response in normal Swiss mice who were recipients of Algire type chambers containing liver cel from immunized donors. Bovine serum albumin (BSA) was selected as antige because it can be made particulate by incorporating it into hemoglobin-deoxyribonucleic acid particles (Nelson, 1958) thereby insuring its uptake by phagocytic cells. Swiss mice of the strain employed (Curd's Caviary and Animal Supply, La Puente, California) are not sensitized by a single I injection of soluble BSA but do respond to the antigen when incorporated hemoglobin particles.

Experimental

Donor mice were injected IP with 0.6 mg BSA in hemoglobin particles. Twenty-one days later the animals were bled to death and their livers removed to ice-cold Hank's balanced salt solution. The tissue was homogenized in a Kontes glass tissue grinder and centifuged at 500 g for 10 minutes. The cell suspension (0.4 ml) was placed into 1.2 x 0.5 cm lucite chambers with Millipore filters (0.30 micron pore size) on either side. These were inserted into the peritoneal cavaties of normal mice. Four days later the recipients were given an IP injection of 0.2mg BSA in saline. Eight days after this booster injection (day 12) the mice were bled and their sera tested for antibody to BSA by the ammonium sulfate precipitation technique of Farr (1958) (See Table I). Antibody was determined by comparing with the test sera the percentage of radioactivity precipitated by similar dilutions of serum from normal mice. In a series of tests on pools of serna from several hundred normal mice the greatest amount of precipitated radioactivity ever found at a 1:5 dilution was 12%. Values usually were 8 to 10 percent. A serum consistently precipitating more than 12% of a standard

amount of antigen at a 1:5 dilution was regarded as having some antibody.

TABLE I

Antibody Response to BSA in Chamber Recipients

Number of Mice	Received	Booster (0.2 mg BSA IP)	Bled	%Radioactivity	Serum Dilution
5	Liver in chamber	day 4	day 12	99	1:5
				99	1:10
				98	1:20
				97	1:40
				81	1:80
				57	1:160
				33	1:320
				22	1:640
5	Liver in chamber	none	day 7	9	1:5
				7	1:10
6	Liver IP no chamber	day 4	day 12	9	1:5
				7	1:10
6	Liver no chamber	none	day 8	8	1:5
				6	1:10

Control animals received intraperitoneal injections of the same number of liver cells as were inserted in the chambers. One group was bled at eight days and the other was given a booster injection (0.2 mg BSA in saline IP) at four days and bled eight days later (day 12). Neither of these groups developed antibodies to BSA.

Upon removing chambers at various times and examining the contents it was found that liver cells did not survive more than a day or two. Such limited survival of cells in chambers may mean that viability of the liver cells in our experiments is not a requirement for transfer of the "primer" for antibody formation. Some indication of this was also found in experiments with chambers containing liver cells refrigerated 24 hours before transfer. In this experiment (Table II) the recipients of refrigerated as well as fresh liver produced some antibody.

Unsuccessful attempts were made to initiate antibody formation with liver cells from intravenously sensitized mice. This route of sensitization in mice previously had been shown by Nelson (1957) to be ineffective for BSA in hemoglobin particles because the particles are filtered out in the lungs.

TABLE II

Antibody Response to BSA in Recipients of Fresh and Refrigerated Liver Cells in Chambers

Number of Mice	Received	Booster (0.2 mg soluble BSA IP)	Bled	%Radioactivity Precipitated	Serum Dilution
6	Fresh Liver in Chamber	day 4	day 12	23 17 13	1:5 1:10 1:20
3	Fresh Liver in Chamber	none	day 12	11 8	1:5 1:10
9	Liver refrigerated 24 hours in chamber	day 4	day 12	17 12 11	1:5 1:10 1:20
6	Donor serum taken at time of transfer.			10 8	1:5 1:10
6	Donor mice given a booster at time corresponding to 4 days after transfer Bled day 12			76 51 35 20 14	1:5 1:10 1:20 1:40 1:80

Discussion

This study demonstrated that information for antibody formation ("primer") is contained in liver cells of sensitized animals. Apparently other attempts to demonstrate this were thwarted by being limited to unsuccessful searches for primary antibody possibly present but

below a detectable level. The anamnestic response, characterized by a rapid use in antibody upon stimulation by only a small amount of antigen, advantageously permitted us to determine here that information for antibody synthesis had been transferred via the liver.

There are several obvious questions which should be answered here. The antibody found in sera of recipients of sensitized liver cells was not carried over from the donors nor was it the result of stimulation by trapped antigen transferred with the liver cells. Were this so then injection of the liver cells intraperitoneally without a chamber should also have resulted in similar amounts of antibody. Furthermore, donor animals were not actively forming detectable antibody at the time the livers were excised. It seems unlikely also that the transferred liver itself formed antibody in view of the finding that cells inside of chambers die rapidly. That some antibody also developed when refrigerated liver cells were employed is further evidence favoring a hypothesis that the information for antibody is contained in subcellular fractions.

Further work is now in progress to determine the nature of this carrier of information for antibody production.

Acknowledgements

The authors wish to express their appreciation to Mrs. Sandee Cooper for excellent technical assistance.

References

Askonas, B.A., J. H. Humphrey and R. R. Porter, Brit. J. Exptl. Path. 37, 61 (1956)
Farr, R. S., J. Inf. Dis. 103, 239 (1958)
Garvey, J. S. and D. H. Campbell, J. Immunol. 76, 36 (1956)
Garvey, J. S. and D. H. Campbell, J. Exptl. Med. 105, 361 (1957)
Halpern, B. N., J. Pharm. and Pharmacol. 11, 321 (1959)
Miller, L. L. and W. F. Bale, J. Exptl. Med. 99, 125 (1954)
Nelson, E. L., Fed. Proc. 16, #1833 (1957)
Nelson, E. L., J. Exptl. Med. 107, 769 (1958)
Rittenberg, M. and E. L. Nelson, Am. Nat. 94, 321 (1960)