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Induction of Interferon in Hereditarily Asplenic Mice With and Without a Neonatal Spleen Cell Transplant[1, 2] (38129)

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Several investigations have been made concerning the role of the spleen in the production of virus-induced interferon. Splenectomized mice have been observed to have a reduced ability to synthesize interferon (1–3). However, other investigators have reported that splenectomy has no effect on virus-induced interferon production in mice (4), rats (5), or rabbits (6). The availability of a colony of hereditarily asplenic mice provided a unique experimental model for further study on the function of the spleen in interferon synthesis.

Materials and Methods. Mice. An inbred colony of hereditarily asplenic mice carrying the gene for dominant hemimelia (Dh) was used as the source of test animals. The colony is maintained at the Memorial Research Center and has been described in earlier publications (7–9). The mice were used at 2 mo of age. Thirty heterozygous (Dh/+) mice constituted the experimental groups and an equal number of homozygous (+/+) normal littermates were used as controls.

Neonatal spleen cell transplants. Spleen cells were obtained from one month old normal donors (+/+) and 20 × 10^6 cells, in 0.1 ml of saline, were injected subcutaneously into both asplenic and normal littermates within 20 hr after birth. The number of spleen cells to be injected was determined on the basis of the number of nucleated cells in the spleen of a newborn mouse, which ranged from 15–23 × 10^6 cells. These mice were then held for 2 mo before being used in the interferon experiments. An autopsy was made in all transplanted mice to determine the macroscopic appearance of the graft. Tissue sections were also examined by standard techniques.

Virus strains. The Herts strain of Newcastle disease virus (NDV) has been in our stock collection for many years as a chick embryo allantoic fluid preparation. The Indiana strain of vesicular stomatitis virus (VSV) was obtained from Dr. R. R. Wagner of the University of Virginia as a chick embryo cell culture stock. Both preparations titered 1 × 10^9 plaque forming units (PFU) per ml and maintained this titer in the frozen state at −70°.

Cell cultures. L cells. The CCL-L strain of mouse embryo L cells were obtained from the American Type Culture Collection and were maintained by weekly subculture in Eagle's minimal essential medium (MEM).

Production of interferon test preparations. Each mouse was injected in the tail vein with 0.2 ml (2 × 10^8 PFU) of the NDV preparation. Serum samples were collected at 5, 15, and 24 hr after virus injection and frozen at −20° until assayed.

Assay of test materials. Pooled serum samples from each group of mice were assayed in L cell monolayers using the plaque reduction technique with VSV as the challenge virus (10). The assay method has been previously described (10, 11). Sera from the transplanted and the nontransplanted animals were assayed in duplicate at different times. The pooled serum samples were diluted 1:10 and doubling dilutions were then made from the 1:10

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TABLE I. Serum Interferon Titers of Congenitally Asplenic Mice and Normal Littermates.

Group	(5) Hr After iv Injection of 2×10^8 PFU of NDV	(15)	(24)
Asplenic (Dh/+)			
Untreated	1:320	1:640	1:640
Transplanted[a]	1:2560	1:5120	1:2560
Normal (+/+)			
Untreated	1:5120[b]	1:5120	1:1280
Transplanted[a]	1:2560	1:10,240	1:5120

[a] 20×10^6 spleen cells were given subcutaneously within 20 hr of birth and mice used 2 mo after transplantation.

[b] Each value in the table represents the mean titer of duplicate determinations made in pooled serum samples from 5 to 7 mice.

dilution. As one of the controls, a parallel titration of VSV was run on chick embryo monolayers. The titer of the test preparations was taken as the dilution producing a 50% reduction in the number of plaques.

Results. Ninety-two percent of the mice transplanted with spleen cells at birth developed visible red-purple patches and nodules (1–5 mm in diameter) of splenic tissue at the site of injection and/or the surrounding area two months later. Microscopically, the nodules and patches were largely composed of tightly packed small lymphocytes. Proliferation of granulocytic and erythrocytic precursors and reticular cells was observed scattered throughout the transplanted spleen tissue. Megakaryocytes were occasionally seen.

Endogenous interferon titers were less than 1:10 in both normal and asplenic mice. The titers after injection of NDV are presented in Table I. The normal (+/+) mice had a maximum titer of 1:5120 at 5 and 15 hr whereas the asplenic (Dh/+) mice had a maximum titer of 1:640 at 15 and 24 hr. In this assay, a variation of one doubling dilution is not regarded as significant. A difference of 2 doubling dilutions, however, was considered significant ($P < 0.01$). Thus, the asplenic mice appear to have a markedly impaired ability to synthesize interferon. Homozygous (normal) mice given neonatal spleen cell transplants appeared to have somewhat similar titers of interferon as untreated homozygous mice. The transplanted asplenic mice had much greater titers than their nontransplanted counterparts which, in fact, approached the interferon titers of their normal (+/+) littermates which did not receive a spleen cell transplant. The graft, therefore, restored the capacity of asplenic mice to synthesize nearly normal amounts of interferon.

Discussion. The present report supports previous findings indicating that the spleen plays a major role in the synthesis of virus-induced interferon in the mouse. Fluids from organ cultures of mouse spleens excised at various times after intravenous injection of NDV have been shown to contain significant amounts of interferon (1), and several investigators have demonstrated that splenectomized mice have an impaired interferon production (1–3). Similarly, our data show that hereditarily asplenic mice have lower serum interferon titers compared to normal controls. Furthermore, splenic involvement in virus-induced interferon production is indicated by the enhanced synthesis in asplenic mice given a neonatal spleen cell transplant. Neonatal grafting of spleen cells reconstituted asplenic mice to the extent that they were able to produce approximately as much interferon as untreated littermates with spleen. In this context, it is important to stress that lymphocytes, which presumably produce most of the interferon, were the main proliferation cells within the growing spleen graft.

Other investigators have reported that the spleen is not an important site for the synthesis of virus-induced interferon. Reports that splenectomy has no effect on interferon production might be explained by the use of different hosts (5, 6), viruses (5) or other factors peculiar to the experimental design.

Summary. Interferon production in heredi-

tarily asplenic and normal mice following intravenous injection of Newcastle disease virus was compared. Serum from asplenic mice showed a significantly lower interferon level than normal littermates. A neonatal spleen cell transplant markedly enhanced interferon production in asplenic mice to the extent that they were able to produce amounts of interferon approximately the same as normal littermates with spleen.

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