SUPPRESSION OF INTERFERON SYNTHESIS DURING THE GRAFT VERSUS HOST REACTION IN (CBA x C57BL/6) F_1 MICE

L. M. Mentkevich, O. N. Shcheglovitova,
L. N. Gulyanskii, and A. S. Shevelev

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During the development of the graft versus host reaction (GVHR) in (CBA x C57BL/6) F_1 mice after transplantation of spleen cells from mice of the parental C57BL/6 strain, production of serum interferon induced by intraperitoneal injection of Newcastle disease virus was sharply reduced. Interferon production was reduced and later completely abolished in cultures of bone marrow cells from mice during development of the GVHR. This phenomenon can serve as a criterion of the development of the GVHR.

KEY WORDS: graft versus host reaction; bone marrow; interferon.

The present authors and others have investigated interferon production in various immunological states: after immunization [1], after irradiation [3], and in syngeneic and xenogeneic chimerism [2, 8].

In the investigation described below interferon production was studied in F_1 mice during development of the graft versus host reaction (GVHR) induced by transplantation of spleen cells from mice of one parental strain (C57BL/6).

EXPERIMENTAL METHOD

The experimental model consisted of (CBA x C57BL/6) F_1 mice weighing 18-20 g, into which a suspension of spleen cells from C57BL/6 mice or from F_1 hybrids was injected intraperitoneally in a dose of $1 \cdot 10^8$ in a volume of 0.5 ml. The suspension of spleen cells was prepared by the method described previously [5]. To characterize the development of the GVHR at various times after transplantation the splenic index [11] was determined and the number of nucleated cells was counted in the blood (1 ml) and bone marrow (calculated for one femur). Medullary and splenic interferon were obtained by the method described earlier [10]. To obtain serum interferon the mice were injected intraperitoneally with Newcastle disease virus (NDV), strain H, and 5 h later the animals were exsanguinated. Interferon was titrated in continuous cultures of L cells as described previously [4].

EXPERIMENTAL RESULTS

The GVHR in the mice was characterized by an increase in weight of the spleen, leukopenia, and aplasia of the bone marrow [3]. The splenic index in these experiments on the 9th day of development of the GVHR was 2.2 compared with 0.6 in the control. The number of leukocytes in the blood was reduced about fivefold by the 21st day. A decrease in the number of bone marrow cells began on the 10th-14th day and reached a minimum on the 21st day (6-20 times fewer than in the control).

The results of determination of the serum interferon production in mice after transplantation of spleen cells are given in Fig. 1a. It shows that from the 8th day serum interferon production fell sharply, and later in some of the experiments there was no interferon whatever. After transplantation of syngeneic spleen cells serum interferon production was the same as in the intact animals. An equally marked decrease in serum

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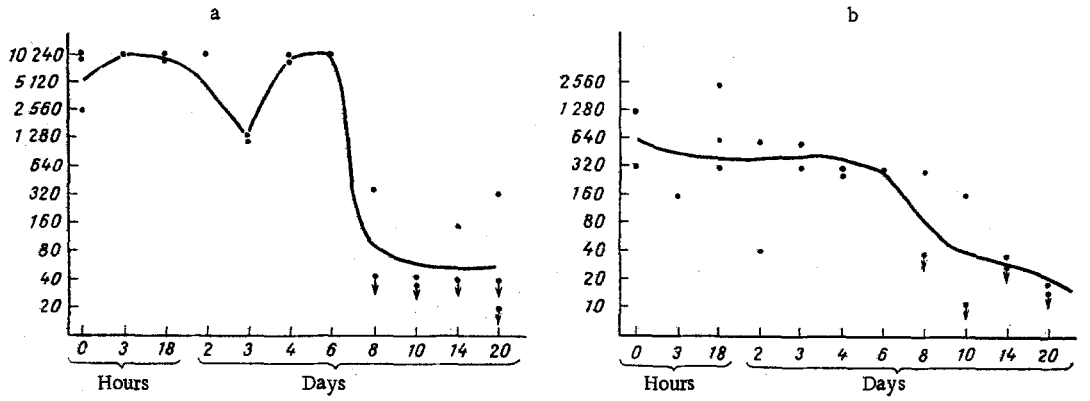

Fig. 1. Production of serum (a) and medullary (b) interferon induced by NDV in F_1 hybrid mice after transplantation of allogeneic spleen. Ordinate, interferon titer; abscissa, time (hours and days).

interferon production was observed only after lethal irradiation of the animals, when the bone marrow was almost completely depopulated and severe leukopenia was observed [2]. In the present experiments only a very small decrease in the number of nucleated blood and bone marrow cells was observed on the 8th day after transplantation, whereas serum interferon production decreased sharply. This fact was surprising, for previous experiments had shown that serum interferon production induced by NDV is brought about mainly by blood cells orginating from the bone marrow [10].

Interferon production in bone marrow cells was induced by NDV after transplantation of allogeneic spleen cells (Fig. 1b). It will be clear from Fig. 1b that on the 8th day after transplantation the ability of the bone marrow cells to produce interferon was sharply inhibited. Later the interferon-producing capacity of the bone marrow cells was minimal, and by the 21st day (period of observation) the bone marrow cells produced no interferon whatever. Evidently as a result of development of GVHR not only was the number of bone marrow cells reduced through the absence of repopulation as the result of death of the stem cells or of the direct "killer" action of immunocompetent cells of the transplanted spleen [3], but the function of the bone marrow cells changed. Changes in the membrane characteristics of the cells during development of the GVHR were probably important [7]. Such bone marrow cells not only produced less interferon themselves or produced no interferon whatever in vitro in response to induction by NDV, but also may have given rise to a population of blood cells which no longer participated in serum interferon production in mice in response to intraperitoneal injection of NDV. Yet another possibility is the changes of this sort took place independently in the blood leukocytes also.

Consequently, the observed decrease in and subsequent absence of serum interferon induced by NDV in (CBA x C57BL/6)F_1 mice after transplantation of spleen cells from the C57BL/6 parents was attributable not only to hypoplasia of the bone marrow and leukopenia, but also to a reduction in and subsequent absence of interferon-producing capacity of the bone-marrow and blood cells as a result of development of the GVHR. The fact that in the early stage of the GVHR, before total manifestations appeared, marked inhibition of interferon production was recorded, to be followed by a complete block in the later stages of the process, is in harmony with results showing the development of immunodepression in the GVHR [11]. The phenomenon observed in these experiments, namely the depression of interferon production, can serve equally with others as a criterion of development of the GVHR.

Since activation of oncornaviruses [6] can be activated during the GVHR and malignant lymphomas [5] can develop, the results now obtained suggest that an essential role in the mechanism of these processes is played by the absence of interferon production in vivo. This hypothesis is in agreement with data showing that injection of interferon into mice can prevent the development of malignant lymphomas [9] and that interferon inhibits replication of mouse leukemia viruses in vitro [6].

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EFFECT OF CONTAMINATION OF GERMFREE GUINEA PIGS BY INDIVIDUAL MEMBERS OF THE INTESTINAL MICROFLORA ON ANTIBODY AND COMPLEMENT LEVELS

V. N. Andreev and G. I. Podoprigora

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On the 3rd day after birth germfree guinea pigs were contaminated by one of the following representatives of the normal intestinal microflora: Bacillus mesentericus, Bacillus subtilis, Staphylococous albus, and Streptococcus faecalis. The levels of antibodies against the microorganisms used for monocontamination and also against Escherichia coli 055, which is pathogenic for guinea pigs, and the serum complement levels were studied in the animals at the age of 2 weeks. Contamination of the guinea pigs by B. mesentericus and B. subtilis did not significantly change the antibody levels against these microorganisms, whereas S. albus and S. faecalis appreciably stimulated antibody formation. Similar results were obtained with respect to E. coli 055. The complement level was significantly increased by the spore-bearing aerobes and by S. albus.

KEY WORDS: germfree animals; normal microflora; antibodies; complement.

Experiments on germfree animals have established that the normal microflora stimulates the immune system of the host [3,4,6]. An interesting aspect of these investigations was the study of the immunogenic properties of individual members of the normal microflora.

The object of this investigation was to study the effect of oral contamination by individual members of the intestinal microflora on antibody formation against hose microorganisms and against Escherichia coli 055, and also the serum complement level of the germfree guinea pigs.

EXPERIMENTAL METHOD

Germfree and monocontaminated animals (6-10 in each group) aged 2 weeks were used.

Germfree guinea pigs were obtained from the mothers toward the end of gestation by caesarian section by a sterile isolation technique [3]. The animals were reared on the BMS-1 sterile diet developed in the writers'

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