# STATUS OF THE T- AND B-LYMPHOCYTES IN LONG-LIVING CBA → $F_1$(CBA × C57BL/6) CHIMERAS

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Semiallogeneic chimeras were obtained by injecting $3 \cdot 10^7$ spleen cells from CBA mice into lethally irradiated $F_1$(CBA×C57BL/6) mice, into which cyclophosphamide was injected two days later in a dose of 2 mg per mouse. This last procedure prevented death of the recipients from the graft versus host reaction (GVHR). In the course of 1.5-2 months complete chimerism of lymphoid tissue, accompanied by specific areactivity of T-lymphocytes relative to the recipient's transplantation antigens was observed in nearly all recipients. Lymphocytes of the chimeras did not react to C57BL/6 antigens either in a mixed culture of lymphocytes or in the local GVHR and did not destroy C57BL/6 cells in vitro. The serum of the chimeras contained blocking factor, preventing the local GVHR. By the third to fifth month lymphocytes of donor origin were beginning to be replaced by recipient's cells, immunoreactivity to the recipient's transplantation antigens was partially restored, and blocking factor disappeared from the blood of the chimeras. KEY WORDS: radiation chimeras; immunologic tolerance; T- and B-lymphocytes; serum blocking factor; graft versus host reaction.

Previous investigations have shown [3, 4] that by injecting cyclophosphamide (CP) into $F_1$(CBA × C57BL/6) mice, after preliminary lethal irradiation and protection by hematopoietic cells and lymphocytes from CBA mice, lethal homologous disease can be prevented and prolonged chimerism of the recipients, lasting several months, can be obtained.

The object of this investigation was to study the immunologic status of such chimeras by the use of tests detecting the parentage and functional activity of the T- and B-lymphocytes.

## EXPERIMENTAL METHOD

Male and female mice of strains CBA and DBA/2 and $F_1$(CBA × C57BL/6) hybrids were used. To obtain mouse chimeras, $F_1$(CBA × C57BL/6) hybrids weighing not less than 30 g were lethally irradiated in a dose of 950 R, after which they were given injections of $3 \cdot 10^7$ CBA mouse spleen cells. Two days later all the animals received an injection of 2 mg CP per mouse. The state of chimerism was evaluated starting from 1.5 months after its creation. The number of antibody-forming cells (AFC) in the spleens of the mice was determined by Jerne's local hemolysis test 2 and 5 months after the creation of chimerism. The mouse chimeras were immunized initially by intravenous injection of $1 \cdot 10^6$ sheep's red blood cells (SRBC) and 4 days before Jerne's test $5 \cdot 10^8$ SRBC were injected intravenously into the animals. The direct test for the presence of chimerism for B-lymphocytes was the method of discriminative analysis of cell suspensions suggested previously [2] by means of strain-specific antiserum. A decrease in the number of AFC as a result of treatment with CBA anti-C57BL/6 serum pointed to their recipient ($F_1$) origin, whereas resistance to such treatment indicated their donor (CBA) origin.

The parentage of the spleen cells of the chimeras also was assessed by the lymphocytotoxic test with CBA anti-C57BL/6 serum. Cells killed by the serum were detected by staining with trypan blue [1].

The functional powers of the T-lymphocytes of the chimeras were determined by means of several tests: their killer activity [7], ability to produce blast transformation in a mixed lymphocyte culture, and ability to

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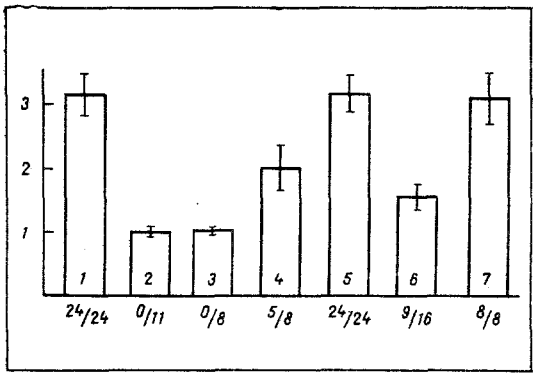


Fig. 1. Intensity of local GVHR given by spleen cells of CBA → $F_1$(CBA × C57BL/6) chimeras. 1) Intact CBA; 2) intact $F_1$(CBA × C57BL); 3) 2-month CBA → $F_1$(CBA × C57-BL/6) chimeras; 4) 5-month CBA → $F_1$(CBA × C57BL/6) chimeras; 5) intact CBA + serum of intact $F_1$(CBA × C57BL/6); 6) intact CBA + serum of 2-month chimeras; 7) intact CBA + serum of 5-month chimeras. Abscissa, number of animals (numerator shows those with positive reaction, denominator total number of animals); ordinate, intensity of GVHR (index of reaction).

give a local graft versus host reaction (GVHR) [12]. To assess blast transformation of lymphocytes in a mixed culture, mouse spleen cells ($5 \cdot 10^6$/ml) were cultured in vitro in RPMI-1640 medium with the addition of 5% embryonic calf serum, $5 \cdot 10^{-3}$ M Hepes, 2 mM glutamine, and $3 \cdot 10^{-5}$ M 2-mercaptoethanol. The tests were carried out in siliconized penicillin flasks (2 ml of cell suspension per flask) and the cells were incubated for 4 days at 37°C. In some experiments the stimulating cells were irradiated in a dose of 1500 R and mixed with responding cells in the ratio of 7:3. Thymidine-$^3$H was added to the cultures in a dose of 4 $\mu$Ci per flask 4 h before the end of incubation. The specific activity of the thymidine-$^3$H was 1 Ci/mmole. The intensity of blast transformation was assessed from the incorporation of thymidine-$^3$H into DNA of the dividing cells, measured with a Packard scintillation counter. To estimate the intensity of proliferation of the lymphocytes quantitatively, the stimulation index ($I_{st}$) was calculated as the ratio a/b, where a is the number of counts per minute in the experimental cultures and b the corresponding number in the control cultures.

To perform the local GVHR test, spleen cells from CBA, $F_1$(CBA × C57BL/6), or chimera mouse cells were injected into $F_1$(CBA × C57BL/6) mice aged 6-8 weeks in a dose of $1 \cdot 10^7$-$1.5 \cdot 10^7$ cells in a volume of 0.2 ml, subcutaneously into a hind-limb footpad. To test the serum for the presence of blocking factor the same number of cells in a volume of 0.1 ml was mixed with 0.1 ml of the test serum and the ability of the suspension to perform the GVHR was studied. The intensity of the reaction was determined seven days later as the ratio between the weight of the regional popliteal lymph node and the weight of the contralateral node (the GVHR index). Suspension was carried out with an accuracy of up to 0.1 mg on an electric analytical balance.

## EXPERIMENTAL RESULTS

In the lymphocytotoxic test, strain-specific CBA anti-C57BL/6 serum did not cause death of the spleen cells obtained from 12 chimeras 1.5 months after the creation of chimerism. Consequently, the spleens of these chimeras contained cells of only donor's (CBA) origin.

Meanwhile, individual testing of the parentage of the AFC in Jerne's test with discrimination showed that in 23 of 26 chimeras tested all the AFC were resistant to the action of CBA anti-C57BL/6 serum, i.e., cells of donor origin also were represented. Partial chimerism was discovered in the other three cases.

A different situation was observed 5 months after the creation of chimerism. At that time, none of the nine chimeras tested was complete; on average only 45% of the AFC consisted of cells of CBA origin.

During the first two months after the creation of chimerism, T- and B-lymphocytes of donor origin only

TABLE 1. Response of Cells of CBA → $F_1$(CBA × C57BL/6) Chimeras in Mixed Lymphocyte Culture

| Series No.* | Group No. | Responding cells | Stimulating cells | Duration of chimerism: 1 1/2 months — No. of counts per min | 1 1/2 months — Index of stimulation | 3 months — No. of counts per min | 3 months — Index of stimulation |
|---|---|---|---|---|---|---|---|
| I | 1 | Chimeras | — | 1872±181 | 0,2 | 14906±1424 | 1,9 |
| | 2† | Intact CBA | — | 9980±506 | 1,0 | 7750±598 | 1,0 |
| II | 3 | Chimeras | $F_1$(CBA×C57BL/6) | 1095±234 | 1,0 | 6375±2858 | 3,1 |
| | 4‡ | » | CBA | 1126±217 | 1,0 | 2063±29 | 1,0 |
| | 5 | » | DBA/2 | 19747±5067 | 17,5 | 41580±2711 | 20,1 |

* In the experiments of series I, 10 million living spleen cells were cultured in 2 ml medium without the addition of stimulating, irradiated cells (monoculture), in series II, 3 million living, responding cells in a volume of 0.6 ml were mixed with 7 million irradiated, stimulating cells in a volume of 1.4 ml (mixed culture).
† Control group for group 1.
‡ Control group for groups 3 and 5.

thus live and function in the recipient. Among these cells there were no lymphocytes capable of reacting to the recipient's antigens. On individual testing of nine chimeras aged two months in no case were T-killers destroying $F_1$(CBA × C57BL/6) target cells found. Cells of the chimeras likewise did not give a local GVHR when injected into $F_1$ mice (Fig. 1).

Data in good agreement with these results were obtained when the activity of the T-lymphocytes of the chimeras was studied in a mixed lymphocyte culture (Table 1). Spontaneous blast-transformation of cells of 1.5-month chimeras was much less marked than in the control during culture of cells from intact CBA. CBA lymphocytes, present in a $F_1$(CBA × C57BL/6) recipient, evidently do not react to its antigens. The results given in Table 1 (groups 3, 4, and 5) confirm this hypothesis. Lymphocytes of mouse chimeras with a period of chimerism lasting 1.5 months were not stimulated by cells from $F_1$ mice (Table 1, group 3). Their proliferative activity (1095 cpm) was virtually indistinguishable from that of the control – group 4 (1126 cpm), in which cells of CBA → $F_1$(CBA × C57BL/6) chimeras were cultured with irradiated CBA cells. Meanwhile normal proliferative activity in response to a "side" antigenic stimulus, namely irradiated cells of the third strain DBA/2 – was preserved in these chimeras (Table 1, group 5).

Parallel with the loss of complete chimerism, by 3-5 months partial recovery of reactivity of the donor's cells was observed relative to the recipient's antigens. Of the eight 5-month chimeras, the cells of five chimeras gave a positive reaction in the local GVHR test (Fig. 1). In the lymphocyte culture spontaneous blast-transformation exceeded the control level (Table 1, group 1), whereas in a mixed culture cells of the chimeras began to react to irradiated $F_1$(CBA × C57BL/6) cells (Table 1, group 3).

Parallel with specific areactivity of the cells of the 1.5-2-month chimeras against $F_1$ antigens, a factor was found in their serum which inhibited the reaction of the CBA cells in the local GVHR test. The sera of seven of the 16 chimeras completely blocked the action of cells of intact CBA mice, whereas the serum of 5-month chimeras did not possess blocking activity (Fig. 1).

It can thus be concluded from these results that in the experimental system used the T- and B-lymphocytes of CBA mice survive for a long time (up to 5 months) in $F_1$(CBA × C57BL/6) hybrid recipients. During the first two months after the creation of chimerism, no cells reacting to the recipient's antigens were revealed functionally among the donor's T-lymphocytes, i.e., tolerance of the CBA cells to the recipient's antigens was observed. These findings agree with the results obtained by other workers who studied the mechanisms of creation and maintenance of tolerance induced in newborn mice [13], in long-living radiation chimeras [14], and in allophenic mice [11]. The facts given above also confirm the view that tolerance to transplantation antigens is the result of elimination or inactivation of the corresponding clones of lymphocytes [6, 8]. The problem of the nature and importance of serum blocking factors, whose presence was discovered in many of the systems mentioned above, likewise still remains unsolved. The blocking factor discovered in the present experiments suppressed the local GVHR, i.e., it was detected in vivo, whereas other workers found this blocking activity only in tests in vitro [9, 10, 15].

The nature and role of the serum blocking factor in the maintenance of tolerance in long-living chimeras require further study. Another factor to be taken into account is in general difficult to find [5, 13].

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