# EFFECT OF SUPPRESSOR CELLS ON IMMUNOLOGIC MEMORY FORMATION IN MICE OF DIFFERENT LINES

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A regulatory role of T suppressor cells is now generally accepted [1-3]. It has been shown in particular that after immunization of mice with sufficiently high doses of sheep's red blood cells (SRBC) T cells specifically depressing the immune response of normal recipients appear [8]. Under these circumstances, immune spleen cells (ISC) taken from the donors on the 145th day after immunization were found to have maximal suppressor activity [4]. It is stated in the literature that the suppressor factors of T cells from different lines of mice participate in the depression of the secondary immune response of syngeneic spleen cells *in vitro* [7]. However, the effect of T suppressor cells on immunologic memory formation has been inadequately studied.

The object of this investigation was to study the effect of T suppressor cells on immunologic memory formation to SRBC in mice.

## EXPERIMENTAL METHOD

Experiments were carried out on adult male mice of lines CBA, DBA/2, and C57BL/6 weighing 20-30 g.

The general scheme of the experiments was as follows. The mice serving as donors of spleen cells were immunized intraperitoneally with SRBC in a dose of $5 \cdot 10^8$. The donors were given an intraperitoneal injection of 200 mg/kg cyclophosphamide (CP) 14 days after immunization. Injection of CP in this dose completely suppressed the secondary immune response of

TABLE 1. Effect of Immune Spleen Cells Transplanted into Syngeneic Mice on Development of Immunologic Memory

| Mice acting as donors and recipients of ISC | Number of recipients | Time of injection of ISC before (−) or after (+) immunization, days | Number of AFC in spleen (mean values and confidence intervals) |
|---|---|---|---|
| CBA | 15 | −1 | 37 240 (29 030—47 670) |
| CBA | 46 | +1 | 3 631 (2 432—5 420) |
| CBA | 22 | +2 | 7 194 (6 353—8 147) |
| CBA | 6 | +3 | 27 350 (17 860—41 880) |
| CBA | 9 | +6 | 25 350 (8 790—73 110) |
| CBA (control) | 42 | — | 30 830 (25 060—37 930) |
| DBA/2 | 10 | +1 | 647 (303—1 384) |
| DBA/2 (control) | 12 | — | 2 877 (1 816—4 560) |
| C57BL/6 | 24 | +1 | 21 040 (12 910—45 920) |
| C57BL/6 (control) | 30 | — | 36 480 (38 510—40 930) |

Legend. Here and in Table 2, pooled results of six experiments are shown; ISC were not injected in control.

TABLE 2. Effect of ISC Transplanted into Allogenic Mice on Development of Immunologic Memory

| Mice donating ISC | Mice receiving ISC | No. of recipients | Number of AFC in spleen (mean values and confidence intervals) |
|---|---|---|---|
| CBA | CBA | 30 | 7 551 (5 834—9 550) |
| DBA/2 | CBA | 17 | 9 550 (5 636—16 180) |
| — | CBA (control) | 35 | 39 990 (34 830—45 920) |
| C57BL/6 | C57BL/6 | 24 | 21 040 (12 910—34 280) |
| CBA | C57BL/6 | 22 | 4 395 (2 786—6 934) |
| — | C57BL/6 (control) | 30 | 36 480 (32 510—40 930) |

Legend. ISC were injected 1 day after priming

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the ISC to SRBC but did not reduce the ability of these ISC to induce a suppressor effect [5]. The donor mice were killed 24 h after injection of CP and their spleens were removed. A cell suspension was prepared from the spleens, washed in medium No. 199 with antibiotics, and injected intravenously in a dose of $5 \cdot 10^7$ into recipients which were given an intravenous injection of $10^6$ SRBC at various times before or after injection of the spleen cells. The recipients were reimmunized 7 days after primary immunization, with the same dose ($10^6$). Mice in the control were injected with SRBC only, by the same scheme. On the 4th day after reimmunization the number of antibody-forming cells (AFC) in the spleen was determined by Jerne's method [8]. The results were subjected to statistical analysis and geometric means and confidence intervals were calculated at the $P \leqslant 0.05$ level.

## EXPERIMENTAL RESULTS

The results given in Table 1 show that injection of ISC into syngeneic recipients of the CBA line 1 or 2 days after priming caused suppression of the secondary immune response. Corresponding experiments on DBA/2 mice gave similar results. Injection of ISC from C57BL/6 donors into syngeneic recipients had no effect on the magnitude of the secondary immune response.

Injection of ISC of CBA donors into recipients of the same line 1, 3, or 6 days after primary immunization did not affect the secondary immune response (Table 1).

In experiments in which the donor and recipient mice differed with respect to the H-2 complex injection of ISC from CBA mice into C57BL/6 recipients was found to induce marked depression of development of the immunologic memory. The same effect was observed when ISC from DBA/2 mice were injected into CBA mice.

The data described above thus show that the development of immunologic memory for SRBC in the mice was sensitive to the action of suppressor cells. Similar data were obtained previously in a study of the effect of T suppressor cells on the primary immune response to SRBC in mice [4, 8]. Just as in the primary immune response, suppression of immunologic memory formation could be obtained only if ISC were injected in the early period after the antigen (in the present experiments the 1st or 2nd day, but not on the 3rd or 6th days). A further analogy between the two phenomena can be seen when the levels of immunosuppression in mice of different lines are compared: in both cases C57BL/6 mice were more resistant to the suppressor effect of syngeneic ISC than were mice of the CBA and DBA/2 lines.

However, suppression of immunologic memory development differed significantly from suppression of the primary immunologic response, as was revealed when an allogeneic donor–recipient combination was used. ISC from DBA/2 mice, unable to depress antibody formation in CBA mice in response to injection of the optimal dose of SRBC [4, 5], effectively suppressed the development of immunologic memory in this combination. Further investigations are needed to discover the causes of this difference.

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