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Abstract

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KEYWORDS: graft-vresus-host reaction, suppressor cells, CBF1, BALB/C

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SPECIFIC SUPPRESSION OF THE LOGAL GVH REACTION BY F_1 SPLEEN CELLS

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Abstract. The local graft-versus-host (GvH) reaction in (C57BL/6 × BALB/c) F_1 hybrid mice, assayed by popliteal lymph node enlargement, was specifically depressed by an injection of parental lymphocytes mixed with spleen cells from F_1 mice pretreated with the same parental lymphocytes. Suppressor activity of CBF_1 spleen cells was obtained 7 days after inoculation of parental lymphocytes, and peaked on day 10. The suppressive activity was induced by only spleen cells from CBF_1 which was inoculated Balb/c lymphocytes, but not C57BL/6 lymphocytes. The lymphocyte subpopulation responsible for the suppressive activity was noticed in T cell population.

Key words : graft-versus-host reaction, suppressor cells, CBF_1, BALB/c.

Introduction of lymphocytes from the parental strain into F_1 hybrid strains induces a graft-versus-host-reaction (GvHR) which frequently leads to a life-threatening situation. According to classical immunological theory, the hybrid recipient is unable to respond to the injected cells because it has inherited all the cell surface antigen of both parents and expresses them in a co-dominant fashion. But, grafted paternal lymphocytes are able to respond to the host lymphocytes expressed maternal histocompatibility antigens and vice-versa.

Recently, it has been shown that prior treatment of F_1 rats with parental lymphocytes can depress the subsequent GvH reaction, namely, lymphnode hypertrophy due to the local GvH reaction and death due to the systemic GvH reaction are inhibited, (1-4).

The induction of resistance to the GvH reaction required T cells that have reactivity to antigens of the F_1 host. The specific resistance of the F_1 host to the systemic GvH reaction was mediated by host T cells, and could be transferred adoptively to naive syngeneic F_1 host, (2).

In the present experiment, mice of which MHC antigen have been well analysed were used to investigate the resistance of the F_1 host to the GvH reaction. This study shows that inoculation of parental lymphocytes into F_1 mice induced suppressor activity in F_1 spleen cells which can depress the local GvH reaction induced in naive syngeneic F_1 mice by injecting corresponding parental lymphocytes.

MATERIALS AND METHODS

Experimental animals. BALB/c, C57BL/6, C_3H/He, (C57BL/6 × BALB/c) F_1 hybrid (CBF_1) and (C_3H/He × BALB/c) F_1 hybrid (C_3C F_1) mice, were obtained from Shizuoka Agricultural Cooperative Association for Laboratory Animals. The male mice used were between 7 and 8 weeks old.

Preparation of spleen cells. The spleen was removed aseptically, minced in phosphate buffer solution (PBS), then passed through No. 150 wire-mesh. After the contaminated red blood cells were hemolysed with 0.75% ammonium chloride tris solution (0.017 M, pH 7.65), cells were washed three times with PBS, and adjusted at 8×10^8 cell/ml. Trypanblue staining showed that the viability was greater than 90%.

Separation of plastic petri dish adherent cell. Plastic petri dishes were treated with RPMI 1640 supplemented with 10% fetal calf serum for 24 h. Then the cell suspension in the dishes was incubated for 1 h at 37℃ in 5% CO_2 atmosphere. The spleen cells were separated on the basis of there adhesive ability. This procedure was repeated three times.

Treatment with anti-Thy 1.2. antibody. CBF_1 spleen cells were incubated with a 1 : 5000 diluted solution of anti-Thy 1.2. antibody (Olac) for 30 min at 4℃. The cells were then washed and incubated with a 1 : 5 dilution of quinea pig complement for 30 min at 37 ℃, then washed three times.

Treatment with mitomycin C (MMC). CBF_1 spleen cells were incubated in RPMI 1640 solution containing 25 μg/ml of mitomycin C (Kyowa Hakko Kogyo Co. Ltd., Tokyo, Japan) for 30 min at 37℃, then washed three times.

Induction and measurement of the graft-versus-host reaction (GvHR). Using the method of Ford (5), one group of ten CBF_1 mice were injected subcutaneously with 2×10^7 parental strain spleen cells in 0.025 ml into the left footpad of the hindleg, and the same number of CBF_1 mouse spleen cells into the right. On the seventh or eighth day, the wet weight of the bilateral popliteal lylmph nodes (P.L.N.) was measured and used to express the extent of the GvHR as a stimulation index (S.I.).

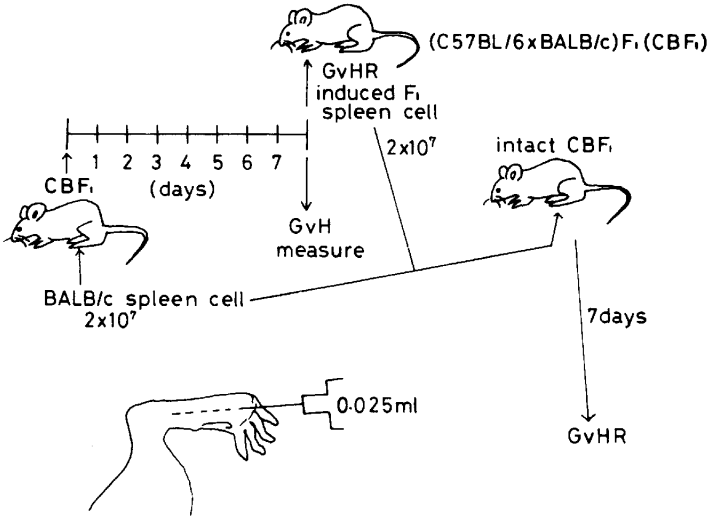

Fig. 1. Schema of experimental system.

S.I. = Wet weight (mg) of P.L.N. of mice injected parental strain spleen cells / Wet weight (mg) of P.L.N. of mice injected of CBF_1 mouse spleen cells.

Measurement of inhibition of the GvH reaction. 2×10^7 parental spleen cells were mixed with the same number of spleen cells (whole cells, fractionated cells MMC treated cells) from CBF_1 mice in which a GvH reaction had been induced. The mixture was injected subcutaneously into the footpad of the hindleg of naive CBF_1 mice to induce a GvH reaction. After 7 days, the S.I. was measured and was compared to that of the control group injected with 2×10^7 cells each of parental strain mouse spleen cells and normal CBF_1 spleen cells (Fig. 1).

RESULTS

Time course of suppressor activity of spleen cells from CBF_1 mice in which the GvH reaction had been induced. The S.I. for the GvH reaction after injection of BALB/c mouse spleen cells into CBF_1 mice increased parallel with injected cell numbers (Fig. 2). The GvH reaction after the injection of 2×10^7 BALB/c mouse spleen cells reached a maximum on the 10th day after injection, and decreased thereafter (Fig. 3). When spleen cells from these GvHR induced CBF_1 mice were injected into the footpad of the hindleg of naive CBF_1 mice together with BALB/c spleen cells (2×10^7 cells of each), the GvH reaction was not suppressed significantly on the third day, but on the 7th, 10th and 14th days 40-50% inhibition was observed. Thereafter, assessments of the inhibition of the GvH reaction by CBF_1 spleen cells were performed on the 7th day (Fig. 3).

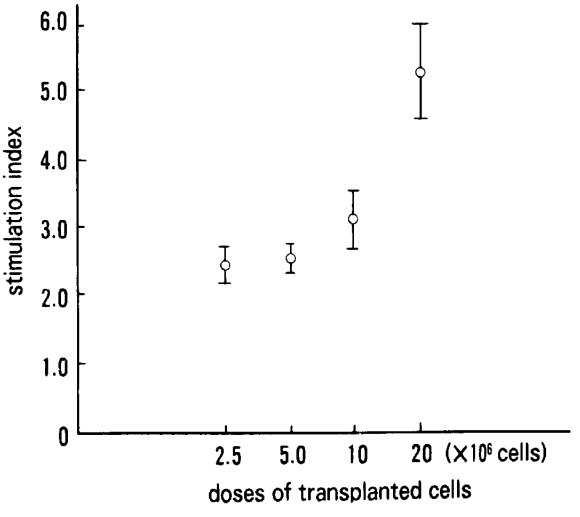

Fig. 2. Doses response of GvHR induced with BALB/c spleen cells.

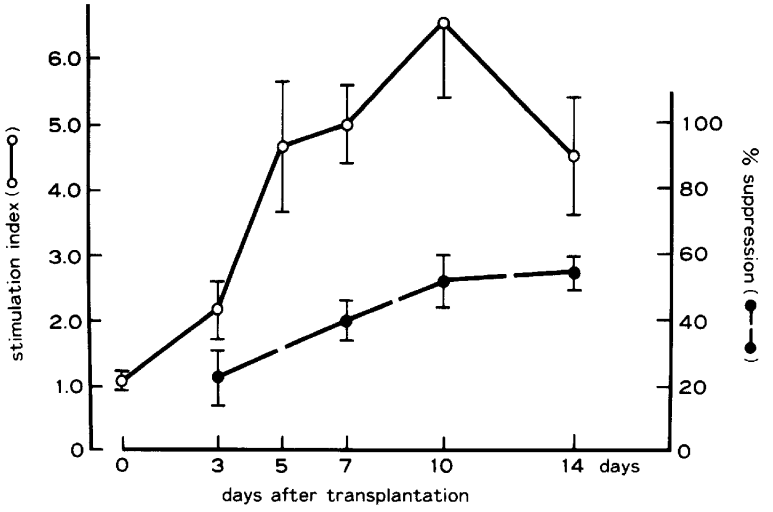

Fig. 3. Relationship of GvHR and GvHR suppression. (○) GvHR induced with BALB/c spleen cells; (●) GvHR suppression by GvHR induced CBF_1 spleen cells.

Characteristics of the induced suppressor cells found amongst CBF_1 spleen. CBF_1 mouse spleen cells on the 7th day after inoculation of BALB/c mouse spleen cells were fractionated in various ways to determine whether the suppressor cells were macrophage or T cell. The results showed that (a) when separated into plastic dish adherent and nonadherent fractions, the latter were most effective in suppression and (b) when plastic dish adherent cells were further treated

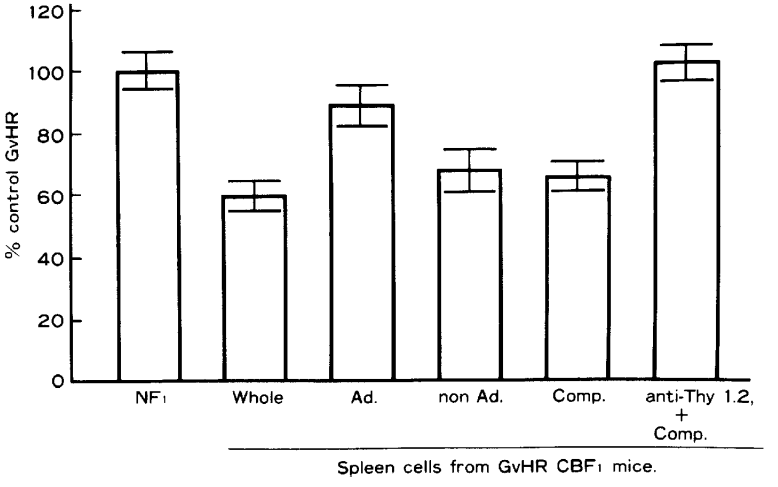

Fig. 4. GvHR suppression by fractionated spleen cells from GvHR induced CBF_1 mice. NF_1: Normal CBF_1 spleen cells, Whole: Whole cells, Ad: Plastic petrie dishes adherent cells, non-Ad; Non adherent cells, Comp: Complement, anty-Thy 1.2.: Anty-Thy 1.2. antibody.

with anti-Thy 1.2. antibody and complement, the suppressor activity was abolished (Fig. 4). This suppressor activity was not lost after treatment with mitomycin C ($p < 0.05$) (Fig. 5). These findings clearly indicate that the relevant cell population mediating GvH suppression consists of T cells.

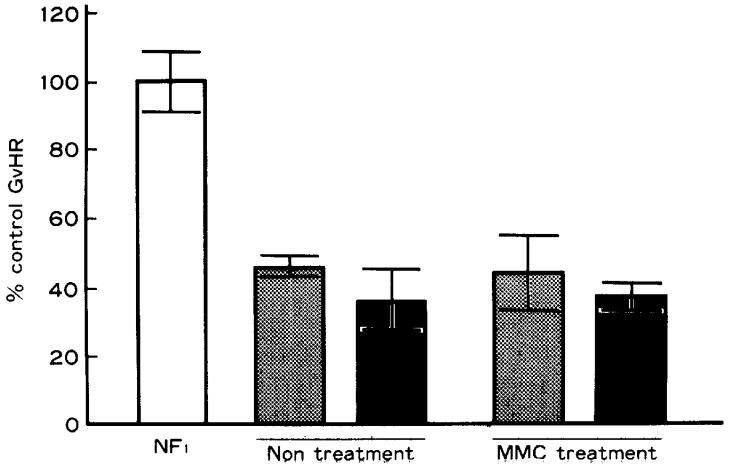

Fig. 5. Effect of MMC on suppressor cells. ☐: 2×10^7 BALB/c spleen cells were mixed with 2×10^7 normal CBF_1 spleen cells; ▨: 2×10^7 BALB/c spleen cells were mixed with 2×10^7 CBF_1 spleen cells induced GvHR with 2×10^7 BALB/c spleen cells; ■: 2×10^7 BALB/c spleen cells were mixed with 2×10^7 CBF_1 cells induced with 4×10^7 BALB/c spleen cells.

Specificity of the GvH reaction inhibition. Specificity of the suppression was

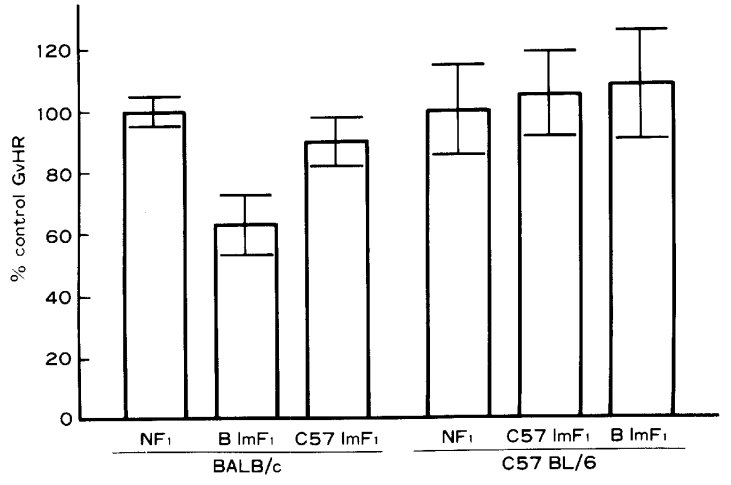

Fig. 6. Specificity of GvHR suppression. NF_1: Normal CBF_1 spleen cells, $BImF_1$: CBF_1 spleen cells induced GvHR with BALB/c spleen cells, $C57ImF_1$: CBF_1 spleen cells induced GvHR with C57BL/6 spleen cells.

demonstrated as follows: Spleen cells from CBF_1 mice in which the GvH reaction had been induced by BALB/c spleen cells inhibited the GvH reaction of BALB/c mouse spleen cells ($p < 0.01$) but did not inhibited the GvH reaction of C57BL/6 spleen cells. Spleen cells from CBF_1 mice in which the GvH reaction had been induced by C57BL/6 mouse spleen cells did not inhibit the GvH reactions of either C57BL/6 or BALB/c mouse spleen cells (Fig. 6).

As mentioned above, GvHR suppressor cells in the spleen of CBF_1 mice were induced by BALB/c spleen cells but not by C57BL/6. Further study was done by the same method using ($C_3H/He \times BALB/c$) F_1 hybrid (C_3C F_1). In this case, GvHR suppressor cells were induced by both BALB/c and C_3H/He parental spleen cell ($P < 0.05$) (Fig. 7).

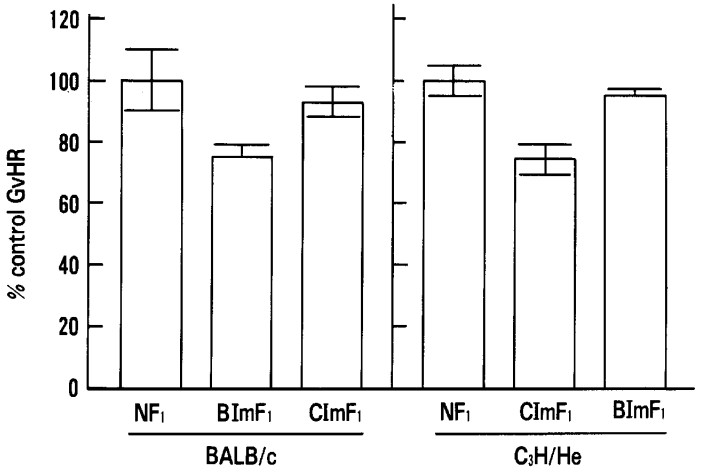

Fig. 7. Specificity of GvHR suppression. NF_1: Normal C_3CF_1 spleen cells, $BImF_1$: C_3CF_1 spleen cells induced GvHR with BALB/c spleen cells, $CImF_1$: C_3CF_1 spleen cells induced GvHR with C_3H/He spleen cells.

DISCUSSION

When the GvH reaction is induced by innoculation with parental strain lymphocytes into F_1 hybrid, immune suppression develops within a few days. This suppression involves a wide spectrum including skin graft rejections (5), antibody production (7, 8), cell mediated lympholysis (CML) related to alloantigens and hapten-modified self antigens (9). These phenomena are nonspecific immunological suppression. In contrast to this, the immunological inhibition in the present experiment of spleen cells from CBF_1 mice undergoing the local GvH reaction was a specific reaction in response to parental lymphocytes which induced GvHR.

Using almost the same method as the present study, Lang (10) reported an investigation of the suppressor cells as follows. A local GvH reaction was in-

duced in rats and, on the 10th day, lymphocytes taken from the F_1 popliteal lymqh nodes were mixed with equal numbers of lymphocytes of the same parental strain for inoculation into naive syngeneic F_1. The subsequent local GvH reaction was strongly inhibited and this suppressor activity was not detected in the F_1 peripheral blood or spleen. In the present study, the inhibitory activity of popliteal lymphnodes in the local GvH reaction was not measured but the sensitized spleen cells suppressed efficiently the local GvHR. The difference between our study and that of Lang are worth considering. Sublethal irradiation was required to induce GvH disease leading to death of F_1 rats, whereas it was not required to induce mortality in F_1 mice. These investigation showed that F_1 rats possessed natural resistance to parental haplotypes.

In the present study, suppressor cells were detected when BALB/c cells were innoculated into CBF_1 mice but not when C57BL/6 cells were innoculated to induce local GvH reaction. Because there was little difference in the stimulation index of the local GvH reactions in these situations, the suppressor activity induced in F_1 spleen was not related to the intensity of the local GvHR. The discrepancy in the suppressive activity could be accounted for by a mechanism that the peripheral blood and spleen of the CBF_1 host has natural resistance to the C57BL/6 mouse H-2^b lymphocytes which prevents sufficient numbers of lymphocytes injected into the footpad from either reaching, or surviving in, the spleen. The genetic pattern of this natural resistance is consistent with the F_1 anti-parent natural resistance to the H-2D-Hh-1 determinant expressed by homozygous H-2^b hemopoietic cells and appearing as F_1 resistance to marrow grafts (11) and systemic GvH (12). Similary, a strong CML occurs with $B6D2F_1$ responder and B6 stimulator, but only a weak CML occurs with D2 stimulator (13). Furthermore, in the study of GvH induced suppressor cell activity for T cell mediated lympholysis to trinitrophenyl modified self and alloantigens, H-2^b parental cells were not able to induce F_1 suppressor cells (14).

What is the nature of suppressor which develop amongst the F_1 host spleen cells in response to the local GvH reaction ? There was no decrease in activity when plastic dish adherent cells were removed, but the activity was lost after treatment with anti-Thy 1.2. antibody and complement. Therefore, it was clear that the cells were T cells. However, it is unclear whether these suppressor T cells arise from the lymphocytes of the parental strain or from the F_1 host. According to Elkins (15), parental strain lymphocytes of F_1 rat undergoing a local GvH reaction in the renal subcapsular region increased in the site of innoculation and in the regional lymph nodes, but did not increase in the spleen. Bergrau (2) showed that immunization of F_1 rats with alloreactive T cell population of parental strain origin induces a host-mediated T cell response which is specific for anti MHC receptors on parental T cells. These results suggest that suppressor T cells for the local GvHR are parental origin. However, in this study, the possibility remains that, in the mouse which has weaker natural resistance than the rat,

lymphocytes inoculated into the footpad migrate to the spleen, and mature into alloantigen-stimulated suppressor cells. In this respect, Rich (14) have reported that alloantigen-activated spleen T cells produce a soluble factor which suppressed mixed lymphocyte reaction proliferative responses, and that a defect in the expression of suppressor activity was identified in the mouse strain C57BL/6.

Thus more detailed study is needed to resolve this question.

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