

# THE Ly PHENOTYPE OF SUPPRESSOR T CELLS ARISING IN MICE SUBJECTED TO A GRAFT-VERSUS-HOST REACTION\*

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Inoculation of parental lymphocytes into  $F_1$  mice results in a graft-versus-host reaction (GvHR)<sup>1</sup>. The humoral immune response in vitro against sheep red blood cells (SRBC) of spleen cells from these "GvH mice" is profoundly suppressed (1-4). On the other hand, the effect of spleen cells from GvH mice on the anti-SRBC response of cultures from untreated mice is H-2 specific and either stimulatory or suppressive, depending on the stage of GvHR. Both activities have been shown to be properties of T cells and to be antigen nonspecific (4). In this study we characterize the T-cell subsets responsible for each effect with respect to their Ly phenotype.

## Materials and Methods

*Mice.* (C57BL/6 × DBA/2) $F_1$  hybrid (BDF<sub>1</sub>) mice were purchased from The Jackson Laboratory, Bar Harbor, Maine. The C57BL/6 congenic strains B6/Ly 1.1 and B6/Ly 2.1 were raised in the Sloan Kettering Institute mouse colony. 6- to 10-wk-old female mice were used.

*Antisera.* Anti-Ly 1.1 serum: (BALB/c × B6) $F_1$  anti-B6 Ly 1.1 thymocytes, absorbed with B6 thymocytes. Anti-Ly 2.2 serum: (C3H × B6/Ly 2.1) $F_1$  anti-B6 leukemia ERLD, absorbed with B6/Ly 2.1 thymocytes. The sera were provided by Dr. U. Hämmerling.

*Cell Cultures.* Mouse spleen cell suspensions were cultured according to Mishell and Dutton (5). The medium contained  $5 \times 10^{-5}$  M 2-mercaptoethanol. Cultures were immunized with SRBC. The antibody response was assayed 4 days later by the Jerne plaque assay as modified by Mishell and Dutton (5).

*Induction of GvHR.*  $8-9 \times 10^7$  viable B6 spleen cells were injected into the tail vein of BDF<sub>1</sub> mice.

*Cytolysis with Antisera and Complement.*  $2 \times 10^7$  cells were incubated in 1 ml anti-Ly serum diluted 1:6, for 30 min on ice, washed, incubated at a cell concentration of  $1.5 \times 10^7$  cells/ml in 1:24 diluted selected rabbit serum as a source of complement for 40 min at 37°C, and washed three times before use. The cells were resuspended in culture medium in a volume corresponding to  $5 \times 10^6$  cells/ml of the complement control.

## Results

To induce GvHR  $8-9 \times 10^7$  viable spleen cells of C57BL/6 mice (Ly 1.2, Ly 2.2) or their congenic partner strains B6/Ly 1.1 (Ly 1.1, Ly 2.2) or B6/Ly 2.1 (Ly 1.2,

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<sup>1</sup> Abbreviations used in this paper: GvHR, graft-versus-host reaction; PFC, plaque-forming cells.

Ly 2.1) were injected into BDF<sub>1</sub> mice (carrying both alleles of Ly 1 and Ly 2). Spleen cells of these GvH mice were removed 3 or 7 days ("GvH 3 days", "GvH 7 days") after induction of GvHR, treated with anti-Ly antisera and complement, and transferred into culture. They were tested for their function as helper or suppressor cells in vitro by titration with spleen cell cultures of untreated BDF<sub>1</sub> mice or as indicated otherwise. Cultures were immunized with SRBC. The generation of plaque-forming cells (PFC) was determined after a culture period of 4 days.

*GvH 3 Days Spleen Cells.* 3 days after inoculation of B6/Ly 1.1 spleen cells into BDF<sub>1</sub> mice the spleen cells of these GvH 3 days mice are totally suppressed in their ability to form antibodies against SRBC in vitro. Elimination of Ly 1<sup>-2+</sup> and Ly 1<sup>+2+</sup> cells by cytolysis with anti-Ly 2.2 serum and complement abrogated their unresponsiveness, while removal of Ly 1<sup>+2-</sup> and Ly 1<sup>+2+</sup> populations by cytolysis with anti-Ly 1.1 serum and complement showed no visible effect. This could be due to removal of helper T cells and to not having removed the suppressor cells in question. However, a mixture of anti-Ly 1 with anti-Ly 2-treated cells (containing now Ly 1<sup>+2-</sup> and Ly 1<sup>-2+</sup>, but no Ly 1<sup>+2+</sup> T cells) gave a normal immune response. These findings strongly suggest that the T cell responsible for suppression in the GvH 3 days spleen is of Ly 1<sup>+2+</sup> phenotype (Fig. 1).

Though GvH 3 days spleen cells by themselves are suppressed, they act stimulatory when added to untreated BDF<sub>1</sub> spleen cell cultures. This enhancing effect is entirely sensitive to treatment of the cells with anti-Ly 1 serum and complement (Fig. 2 B and D). The participation of a small subpopulation of Ly 1<sup>+2+</sup> cells cannot yet be excluded, but the phenotype of the vast majority of stimulatory cells must be described as Ly 1<sup>+2-</sup>.

*GvH 7 Days Spleen Cells.* 7 days after injection of B6/Ly 1.1 spleen cells into BDF<sub>1</sub> mice, the spleen cells of these GvH 7 days mice by themselves are unable to respond and they exert a strong suppressive effect on antibody formation of untreated BDF<sub>1</sub> spleen cell cultures. This suppression is sensitive to treatment with both anti-Ly sera (anti-Ly 1.1 or anti-Ly 2.2, respectively) and complement (Fig. 3 B). Thus, neither Ly 1<sup>+2-</sup> cells (present in the anti-Ly 2-treated population) nor Ly 1<sup>-2+</sup> cells (present in the Ly 1-treated population) are suppressive in this system. The possible necessity for cooperation between Ly 1<sup>+2-</sup> and Ly 1<sup>-2+</sup> cells is ruled out by the fact that a mixture of GvH spleen cells lysed by anti-Ly 1 with the population lysed by anti-Ly 2 serum and complement did not restore the suppressive capacity. From these findings it must therefore again be concluded that the suppressor cell in the GvH spleen cell is of Ly 1<sup>+2+</sup> phenotype.

*Origin of the Stimulatory and the Suppressive T Cell of GvH Mice.* When B6/Ly 2.1 spleen cells were used to induce GvHR in BDF<sub>1</sub> mice, treatment of the GvH spleen cells 3 or 7 days after induction with anti-Ly 1.1 or anti-Ly 2.2 serum and complement did not diminish their stimulatory or suppressive activities (Fig. 2 A and C, and 3 A). Both sera were directed against Ly antigens expressed on BDF<sub>1</sub> but not on B6/Ly 2.1 T cells. Therefore, a participation of host T cells in either suppression or stimulation by GvH spleen cells is ruled out.

These experiments further provide serological controls for the used anti-Ly sera: induction of GvHR with B6/Ly 1.1 spleen cells resulted in anti-Ly 1.1 and

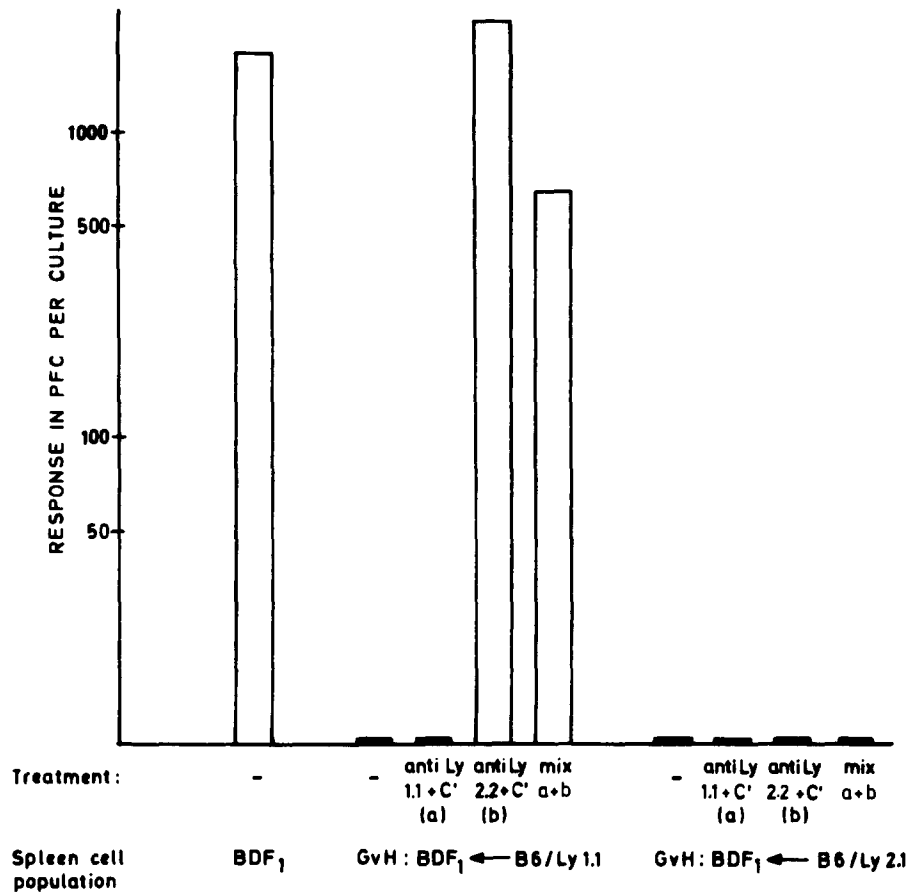


FIG. 1. Immune response against SRBC of spleen cell cultures ( $5 \times 10^6$  cells/ml) from BDF<sub>1</sub> mice 3 days after injection of either B/6 Ly 1.1 or B/6 Ly 2.1 spleen cells. The GvH spleen cells are either untreated or treated with anti-Ly 1.1 (a) or anti-Ly 2.2 (b) serum, respectively, and complement. The mixture of population (a) and (b) consists of equal volumes of both.

anti-Ly 2.2 sensitive activities, while those activities achieved by using B6/Ly 2.1 as donors were not affected by these sera.

### Discussion

Splenic T cells from BDF<sub>1</sub> mice injected with B6 spleen cells (GvH mice) exert two different effects on antibody formation against SRBC in spleen cell cultures from untreated BDF<sub>1</sub> mice: GvH spleen cells taken early after induction of GvHR enhance the response, while those of later stages act suppressively (4). In this study we characterized by serological means the T-cell subsets which are responsible for those effects. Stimulatory T cells were found to be sensitive to lysis with anti-Ly 1 serum, but not to lysis with anti-Ly 2 serum. They thus have the same Ly phenotype, namely Ly 1<sup>+</sup>2<sup>-</sup>, as helper T cells (6-8) and T cells which produce T-cell-replacing factor (9). Suppressor T cells in this system could be eliminated with anti-Ly 1 or anti-Ly 2 antiserum. As mixing of anti-Ly 1 lysed

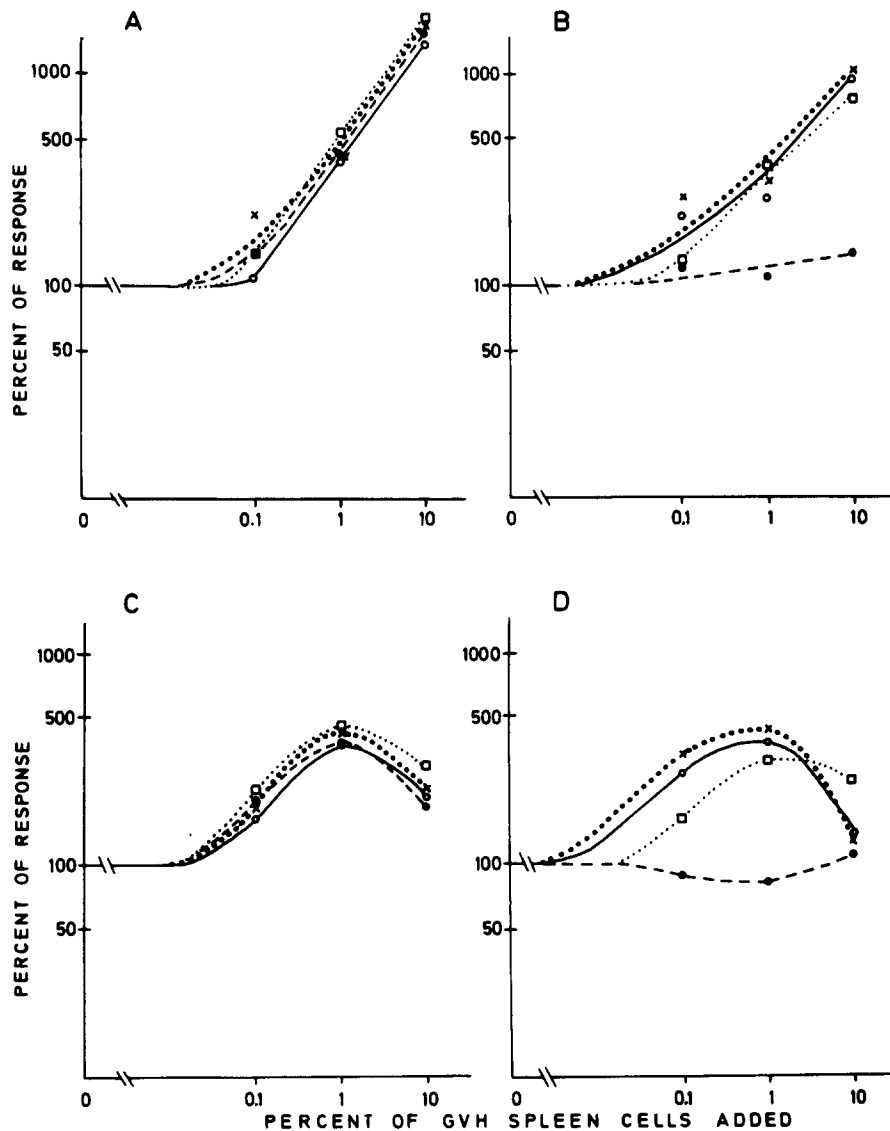


FIG. 2. Titration of GvH spleen cell subpopulations from BDF<sub>1</sub> mice 3 days after graft inoculation with cultures of spleen cells from untreated BDF<sub>1</sub> mice. Cultures contained  $5 \times 10^6$  cells/ml. Anti-SRBC PFC/culture were determined on day 3 (A and B) and on day 4 (C and D) and are plotted as percent of PFC in control cultures to which no GvH spleen cells were added (day 3: 100% = 93 PFC/culture; day 4: 100% = 1,805 PFC/culture). Each point represents the mean of three cultures. (A and C) GvH spleen cells from BDF<sub>1</sub> mice injected with B6/Ly 2.1 spleen cells. (B and D) GvH spleen cells from BDF<sub>1</sub> mice injected with B6/Ly 1.1 spleen cells. (x---x) Untreated GvH spleen cells; (●- -●) treatment with anti-Ly 1.1 serum and complement; (□· · · □) treatment with anti-Ly 2.2 serum and complement; and (○—○) treatment with complement alone.

cells with anti-Ly 2 lysed cells did not reconstitute suppressive activity, a cooperation between those two populations is excluded. Thus, the phenotype of the suppressor T cell in GvH mice has to be described as Ly 1<sup>+</sup>2<sup>+</sup>.

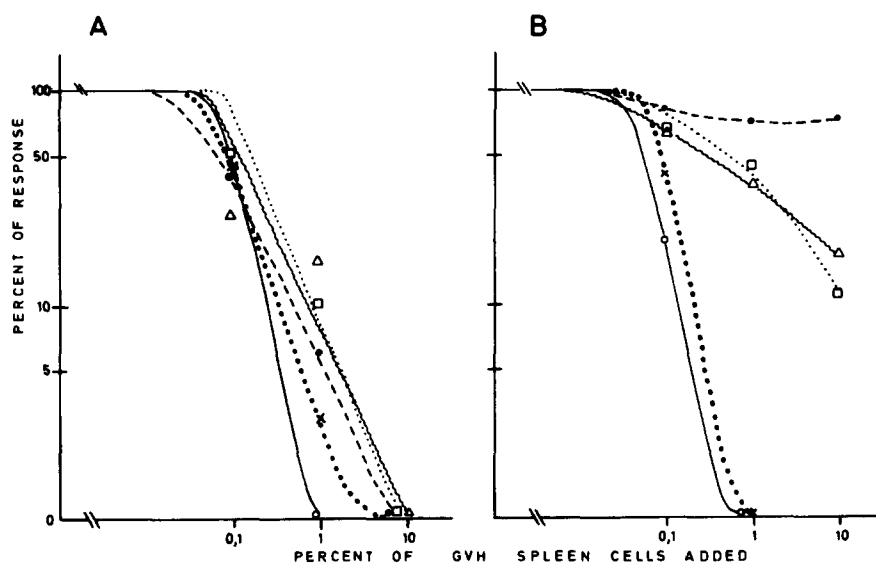


FIG. 3. Titration of GvH spleen cell subpopulations from BDF<sub>1</sub> mice 7 days after graft inoculation with cultures of spleen cells from untreated BDF<sub>1</sub> mice. (A) GvH spleen cells from BDF<sub>1</sub> mice injected with B6/Ly 2.1 spleen cells. (B) GvH spleen cells from BDF<sub>1</sub> mice injected with B6/Ly 1.1 spleen cells. (x●●x) Untreated GvH spleen cells; (●-●) treatment with anti-Ly 1.1 serum and complement (a); (□··□) treatment with anti-Ly 2.2 serum and complement (b); (Δ~Δ) mixture (equal volumes) of populations (a) and (b); and (○-○) treatment with complement alone. Anti-SRBC PFC/culture were determined on day 4 and plotted as percent of PFC in control cultures (3,050 PFC/culture) to which no GvH spleen cells were added.

In other systems suppressor T cells with other properties, e.g. antigen specificity or acting on other cells, have been shown to express no Ly 1 but Ly 2 antigen only (10-13). Thus, there are obviously subcategories of suppressor T cells which express different Ly phenotypes. A similar finding was recently described for cytotoxic T cells (14).

The lack of antibody formation by GvH spleen cells is not due to removal or damage of cell populations necessary for the humoral immune response. The elimination of suppressor T cells from GvH spleen cells, by cytolysis with anti-Ly 2 serum, results in a response against SRBC which is comparable with that of spleen cell cultures from untreated BDF<sub>1</sub> mice. Thus, responsive and suppressive lymphocytes coexist in the spleen of GvH mice. This is also obvious from the finding that GvH 3 days spleen cells by themselves are totally suppressed, while the same cells when titrated with cultures from untreated BDF<sub>1</sub> mice cause stimulation. This seeming discrepancy can be explained by quantitative reasons: there are more helper than suppressor T cells, and, therefore, the latter are diluted out faster. However, the higher amount of helper T cells in the GvH 3 days spleen cell population alone is not able to overcome the inhibition of antibody production caused by a small number of obviously very effective suppressor T cells.

Our findings clearly show that helper and suppressor functions in this system are carried out by distinct subsets of T cells. This is in contrast to the suggestion of Coutinho (15) that suppression is simply due to excess of helper activity. As

there is so far no evidence that T cells which lost a Ly surface antigen regain this marker at a later stage, it seems unlikely that stimulatory cells (Ly 1<sup>+</sup>2<sup>-</sup>) turn into suppressor cells (Ly 1<sup>+</sup>2<sup>+</sup>) during GvHR.

The congenic strain (B6/Ly 2.1) does not express the Ly alleles against which the Ly antisera used in this study (anti-Ly 1.1 and anti-Ly 2.2) are directed. By using those spleen cells for induction of GvHR we showed that host cells (BDF<sub>1</sub>, bearing both alleles of Ly 1 and Ly 2) neither contribute to the enhancing nor to the suppressing effect exerted by GvH spleen cells on the immune response of BDF<sub>1</sub> spleen cell cultures. As the host is tolerant against parental alloantigens its lymphocytes are not activated by the graft. Our finding now excludes the possibility that the environment (e.g., mediators produced by the grafted lymphocytes) in the GvH mouse acts as an inducer of suppressor cells in the lymphocyte population of the host.

### Summary

T cells with helper and suppressor functions arising during graft-versus-host reaction (B6 vs. BDF<sub>1</sub>) have been characterized with respect to their Ly surface antigens. Helper cells were found to express the phenotype Ly 1<sup>+</sup>2<sup>-</sup> and suppressor cells the phenotype Ly 1<sup>+</sup>2<sup>+</sup>. Ly 1<sup>-</sup>2<sup>+</sup> T cells had no suppressive effect in this system. T cells of the host did not contribute to either activity.

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### References

1. Möller, G. 1971. Suppressive effect of graft versus host reactions on the immune response to heterologous red cells. *Immunology*. 20:597.
2. Sjöberg, O. 1971. Antigenic competition *in vitro* of spleen cells subjected to GvH reaction. *Immunology*. 21:351.
3. Sjöberg, O. 1972. Effect of allogeneic cell interaction on the primary immune response *in vitro*. Cell types involved in suppression and stimulation of antibody synthesis. *Clin. Exp. Immunol.* 12:365.
4. Pickel, K., and M. K. Hoffmann. 1977. Suppressor T cells arising in mice undergoing a graft versus host response. *J. Immunol.* 118:653.
5. Mishell, R. I., and R. W. Dutton. 1967. Immunization of dissociated spleen cell cultures from normal mice. *J. Exp. Med.* 126:423.
6. Kisielow, P., J. A. Hirst, H. Shiku, P. C. L. Beverley, M. K. Hoffmann, E. A. Boyse, and H. F. Oettgen. 1975. Ly antigens as markers for functionally distinct subpopulations of thymus derived lymphocytes of the mouse. *Nature (Lond.)*. 253:219.
7. Cantor, H., and E. A. Boyse. 1975. Functional subclasses of T lymphocytes bearing different Ly antigens. I. The generation of functionally distinct T cell subclasses is a differentiative process independent of antigen. *J. Exp. Med.* 141:1376.
8. Feldmann, M., P. C. L. Beverley, M. Dunkley, and S. Kantiainen. 1975. Different Ly antigen phenotypes of *in vitro* induced helper and suppressor cells. *Nature (Lond.)*. 258:614.
9. Pickel, K., U. Hämmerling, and M. K. Hoffmann. 1976. The Ly phenotype of T cells releasing T cell replacing factor. *Nature (Lond.)*. 264:72.

10. Beverley, P. C. L., J. Woody, M. Dunkley, and M. Feldmann. 1976. Separation of suppressor and killer T cells by surface phenotype. *Nature (Lond.)* 262:495.
11. Herzenberg, L. A., K. Okumura, H. Cantor, V. L. Sato, F. W. Shen, E. A. Boyse, and L. A. Herzenberg. 1976. T-cell regulation of antibody responses: demonstration of allotype specific helper T cells and their specific removal by suppressor T cells. *J. Exp. Med.* 144:330.
12. Jandinski, J., H. Cantor, T. Tadakuma, D. L. Peavy, and C. W. Pierce. 1976. Separation of helper T cells from suppressor T cells expressing different Ly components. I. Polyclonal activation: suppressor and helper activities are inherent properties of distinct T-cell subclasses. *J. Exp. Med.* 143:1382.
13. Cantor, H., F. W. Shen, and E. A. Boyse. 1976. Separation of helper T cells from suppressor T cells expressing different Ly components. II. Activation by antigen after immunization, antigen-specific suppressor and helper activities are mediated by distinct T-cell subclasses. *J. Exp. Med.* 143:1391.
14. Shiku, H., T. Takahashi, M. A. Bean, L. J. Old, and H. F. Oettgen. 1976. Ly phenotype of cytotoxic T cells for syngeneic tumor. *J. Exp. Med.* 144:1116.
15. Coutinho, A. 1975. The theory of the "one nonspecific signal" model for B-cell activation. *Transplant. Rev.* 23:49.