Proc. Natl. Acad. Sci. USA
Vol. 78, No. 10, pp. 6441–6445, October 1981
Immunology

Suppressor factor from a T cell hybrid inhibits delayed-type hypersensitivity responses to azobenzenearsonate

(suppressor cells/T hybridomas)

R. BLAKE WHITAKER*, JERRY T. NEPOM*, MAN-SUN SY*, MUNEO TAKAOKI*, COLETTE F. GRAMM*, IRA FOX*, RONALD N. GERMAIN*, MITCHELL J. NELLES†, MARK I. GREENE*, AND BARUJ BENACERRAF*

*Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115; and †Department of Biology, Brandeis University, Waltham, Massachusetts 02154

Contributed by Baruj Benacerraf, July 20, 1981

ABSTRACT **By using polyethylene glycol 1540, BW5147 AKR T lymphoma cells were fused with splenocytes from A/J mice treated so as to induce suppressor T cells specific for azobenzenearsonate (ABA). Of 576 microwells originally seeded, 132 demonstrated growing cell clones, 4 of which produced an ABA-binding supernatant factor. When tested *in vivo* for suppression of delayed-type hypersensitivity to ABA, two of these cell lines, A4 and F12, were shown to produce suppressive supernatant factors. Fluorescence analysis of the F12 cells with appropriate antisera demonstrated this T cell hybrid to be Thy 1.2$^+$, Lyt 1$^+$,2$^-$, and surface immunoglobulin negative, the surface marker phenotype of conventional ABA-specific suppressor T cells. This cloned suppressor cell line, F12, produces a culture supernatant factor that is suppressive at dilutions up to 1:100 and has provided material for genetic and immunochemical analysis.**

Regulation of immune responses involves a complex series of cell–cell interactions and extracellular signals that modulate the type and intensity of response. Several regulatory pathways have been defined in which a specific suppressor T cell or T cell factor inhibits antigen-specific immune cells, in hapten (1–3), polypeptide (4–6), and tumor (7–9) models of murine immune response. One of the most extensively described pathways concerns the suppression of delayed-type hypersensitivity (DTH) to the hapten azobenzenearsonate (ABA) in A/J mice. This suppressor circuit relies on an idiotype–anti-idiotype system of cellular interactions (2). The induction phase of this pathway involves the interaction of antigen with an antigen-binding idiotype-positive T suppressor cell (Ts_1) and relies on an idiotype–anti-idiotype system of recognition. Although the participating T cell subsets and factors are well characterized (1, 2, 10, 11), their complexity has hindered attempts to analyze this suppressor circuit at a molecular level.

Precise analysis of this important regulatory phenomenon requires the isolation of homogeneous suppressor cell populations and factors for detailed study. One approach has been to adapt the hybridoma fusion technique of Köhler and Milstein for use with T cells. Several such fusion products have been generated in various systems and have been reported to: (*i*) have suppressor activity when tested *in vitro* on antigen-specific responses (12–16), (*ii*) bind antigen directly (17, 18), or (*iii*) produce antigen-binding materials (19, 20). This report details the production and characterization of an ABA-specific, suppressor factor-producing, Ts_1-like T cell hybrid line. The hybrid-derived monoclonal factor abrogates the DTH response of A/J mice to ABA and therefore provides a useful reagent for analysis of the molecular basis of suppressor cell function.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Biologicals. Dulbecco's modified Eagle medium with 25 mM Hepes buffer, L-glutamine, glucose at 1 g/liter, and sodium pyruvate was supplemented with 5% 100× antibiotic/antimycotic (DH; GIBCO). Hypoxanthine/aminopterin/thymidine (HAT) components were obtained from Sigma and fetal calf serum was purchased from MA Bioproducts (Walkersville, MD) (lot no. 98115) and was heat inactivated prior to use. BW5147, a Thy 1.1$^+$, T lymphoma cell line of AKR origin that lacks hypoxanthine phosphoribosyltransferase was obtained from the Salk Cell Distribution Center (La Jolla, CA).

Hybridization and Selection. Female A/J mice, 8–10 weeks old, were obtained from the Jackson Laboratory and maintained in our animal facility. Ts_1 ABA-specific suppressor cells were prepared according to the method of Bach *et al.* (1). Briefly, 5 × 10^7 normal A/J spleen cells were derivatized with the diazonium salt of ABA and injected intravenously into nonimmune A/J mice. Recipient mice were splenectomized 7 days later and a single-cell suspension was prepared from their spleens by teasing with forceps and allowing larger fragments to settle out. The splenic lymphocytes were washed twice in 50 ml of phosphate-buffered saline (P_i/NaCl) (pH 7.4) with centrifugation at 250 × *g* for 10 min at 4°C. This cell population was demonstrated to contain ABA-specific suppressor cells by its ability to prevent subsequent DTH response to ABA when transferred to normal A/J mice at the time of initial priming, as described (10).

BW5147 cells maintained in CDH (DH with 10% fetal calf serum) were harvested at the mid-logarithmic phase of growth and washed three times in P_i/NaCl. For fusion, Ts_1 containing A/J splenic lymphocytes were combined at a ratio of 5:1 with BW5147 T lymphoma cells and washed once in P_i/NaCl. The cell mixture was centrifuged at 200 × *g* for 8 min at 4°C, the supernatant fluid was completely aspirated from the cell pellet, and the cells were gently resuspended. The fusogen, polyethylene glycol 1540 [0.3 ml, 40% (wt/vol) in P_i/NaCl; Baker], was added slowly over a period of 45 seconds (21, 22). The cells in polyethylene glycol were held at 37°C in a water bath for 45 sec followed by the addition of 15 ml of P_i/NaCl during a 5-min period. The fused cells were gently pelleted by centrifugation followed by resuspension in CDH. The fused cells then were

Abbreviations: DTH, delayed-type hypersensitivity; ABA, azobenzenearsonate; Ts_1, afferent suppressor T cell; CRI, crossreactive idiotype; DH, Dulbecco's modified Eagle's medium with Hepes buffer; CDH, DH supplemented with 10% heat-inactivated fetal calf serum; P_i/NaCl, phosphate-buffered saline, pH 7.4; HAT, hypoxanthine/aminopterin/thymidine; RIA, radioimmunoassay; FACS, fluorescence-activated cell sorter; GAT, poly(Glu^{60}Ala^{30}Tyr^{10}).

seeded at a density of 6×10^4 BW5147 equivalents per well (0.2 ml) in 96-well, flat-bottomed tissue culture plates (Costar no. 3596, Cambridge, MA). After 24 hr, half the medium was removed and replaced with CDH containing hypoxanthine (100 μM), aminopterin (0.5 μM), and thymidine (10 μM) (CDH/HAT). The cells were fed with CDH/HAT selection medium (23) every second day for 3 weeks, for 1 week with CDH/HT (no aminopterin), and thereafter with CDH alone. HAT selection allows only lymphoma–primary lymphocyte hybrids to survive. BW5147 lacks hypoxanthine phosphoribosyltransferase and is thus HAT sensitive, and primary murine lymphocytes do not survive long-term culture under the conditions employed.

All cells were maintained at 37°C in a humidified 10% CO_2 atmosphere.

Radioimmunoassay (RIA). Culture supernatants from T cell hybrids were assayed for ABA binding material by using a solid-phase competition RIA adapted from Pacifico and Capra (20). Bovine serum albumin ("albumin"; 400 mg) coupled to 100 mg of diazotized arsanilic acid (ABA-albumin) was prepared as described (10). Twenty microliters of ABA-albumin at 200 ng/ml was incubated overnight at 4°C in round-bottomed polyvinyl microtiter plates (Cooke, Dynatech, Cambridge, MA) and removed. After the remaining binding sites on the polyvinyl plates had been saturated by incubation with 1% albumin in P_i/NaCl, supernatants from T cell hybrids were added and allowed to bind for 1 hr at room temperature. ABA groups not bound by supernatant material were then assayed by the addition of 10 ng of monoclonal anti-ABA antibody. The characteristics of this hybridoma protein, R16.7, which expresses the major crossreactive idiotype (CRI), have been described (24). After a further 1-hr incubation, wells were washed and ^{125}I-labeled rabbit anti-mouse immunoglobulin was added for 1 hr to measure bound R16.7 antibody. The amount of ABA-albumin available as a substrate for binding and the amount of R16.7 monoclonal antibody used as a developing antisera were determined by titration to be limiting for ABA-binding sites. Thus if test supernatants contained materials recognizing ABA, they would decrease the amount of R16.7 antibody bound. Any ABA-binding immunoglobulin in the test supernatants registered as noncompeting material because the developing reagent, R16.7, was measured by using ^{125}I-labeled rabbit anti-mouse immunoglobulin.

Antisera and Immunoglobulins. Monoclonal antibodies were used for immunofluorescence analysis of hybrid cells. Antibodies 11.4.1 (anti-K^k) and HO 13.4 (anti-Thy 1.2) were prepared from culture supernatants of cells provided by the Salk Cell Distribution Center. Monoclonal anti-Lyt 1.2 and anti-Lyt 2.2 were the generous gift of F. W. Shen (Sloan-Kettering Memorial Hospital, NY). Preparation of fluorescein-conjugated rabbit anti-mouse immunoglobulin was previously described (25). Affinity-purified rabbit anti-mouse immunoglobulin was radiolabeled for use in RIA by the chloramine-T method (26), using Na^{125}I from New England Nuclear.

Immunofluorescence. T cell hybrids and BW5147 cultured cells were analyzed by indirect immunofluorescence, using a fluorescence-activated cell sorter (FACS II; Becton Dickinson, Mountain View, CA). A pellet of 2×10^6 cells was resuspended in 15 μl of P_i/NaCl containing monoclonal mouse antibodies to H-$2K^k$, Thy 1.2, Lyt 1.2, or Lyt 2.2. After 45 min at 0°C, cells were washed and incubated with fluoresceinated rabbit anti-mouse immunoglobulin at 200 μg/ml for 45 min at 0°C. Cells were fixed with 1% paraformaldehyde in P_i/NaCl prior to FACS analysis.

DTH Assays. Footpad swelling 24 hr after a secondary challenge with ABA served as a measure of DTH reactivity; swelling was measured as described (2). All measurements represent at least four mice per group and were read "blind." Hybridoma supernatants were evaluated for suppressor activity by daily intravenous injection of 0.2 ml of culture supernatant from a growing hybridoma culture (cell density of $5–8 \times 10^5$ per ml) into ABA-primed mice for 5 days, starting the day of priming and ending the day of challenge.

RESULTS

Production of T Cell Hybrids. Six A/J mice were treated intravenously with 5×10^7 ABA-coupled syngeneic spleen cells and challenged 6 days later with ABA diazonium in each of the footpads. When evaluated on day 7, all six mice were negative for DTH to ABA. These mice were splenectomized and the spleens were processed for use as primary lymphocyte parents in the hybridization.

ABA-suppressed A/J splenocytes (180×10^6) were mixed with BW5147 T lymphoma cells (36×10^6) and fused according to the protocol detailed in *Materials and Methods*. The fused cells were resuspended in 116 ml of CDH and seeded at limiting dilution into 576 microwells.

Wells positive for growth appeared as early as 8 days after fusion, and by 20 days after fusion 132 wells (22.9% of the fusion wells) had been selected for expansion and testing. A 100-μl aliquot of supernatant was harvested from each of these wells and assayed for ABA binding by RIA.

Supernatants from T Cell Hybrid Cultures Compete for Antigen Binding. Cell culture supernatants were evaluated for antigen-binding capacity by using a competitive RIA. Of the 132 supernatants tested, 8 (6.1% of growth-positive wells) showed significant inhibition. BW5147 supernatant does not inhibit in this assay and is used as the negative control. Cells from wells containing inhibitory supernatants were expanded in 16-mm wells (Costar no. 3524) and the supernatants once again were tested by RIA for ABA binding. Lines A4, F3, F12, and H11 exhibited the greatest inhibition in this assay and were negative in a similar specific binding inhibition assay using the synthetic polymer poly(Glu^{50}Ala^{40}Tyr^{10}) (GAT). The GAT assay has been used to detect GAT-binding T cell hybrid supernatants (unpublished). The degree of inhibition observed by using supernatants from hybrid lines A4, F3, F12, and H11 is shown in Fig. 1; all inhibition values are expressed relative to the values obtained with supernatant from the BW5147 T lymphoma parent

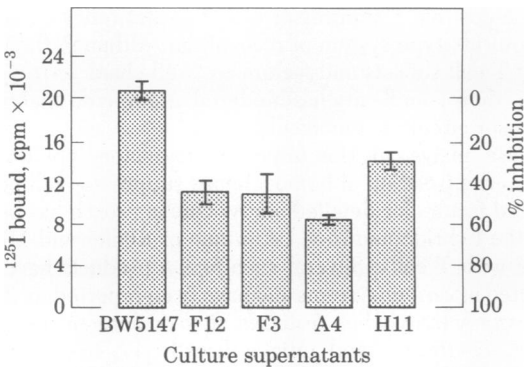

FIG. 1. T cell hybrid supernatants compete in an ABA-binding inhibition RIA. Twenty microliters of culture supernatants from BW5147 T lymphoma or T cell hybrid cultures was added to ABA-albumin-coated microtiter wells, followed 1 hr later by 10 ng of monoclonal mouse anti-ABA antibody. Binding of anti-ABA antibody was assessed by the addition of iodinated rabbit anti-mouse immunoglobulin antibody. Of 132 hybrid supernatants tested, only those from F12, F3, A4, and H11 cultures elicited significant inhibition in this assay. In contrast, the parent T lymphoma cell line, BW5147, did not produce a non-immunoglobulin ABA-binding factor. Error bars indicate SEM.

line. The other 128 supernatants tested did not inhibit the RIA below 70% of the control value. This pattern of inhibition is comparable to that described by Pacifico and Capra (20) with a similar assay, although our frequency of positive hybrids is somewhat lower and the extent of RIA inhibition is slightly less.

Aliquots of these ABA-specific T cell hybrid lines were cryopreserved in liquid nitrogen, and lines F3 and F12 have been maintained as continuous 10-ml cultures (Corning no. 25100, Curtin–Matheson Scientific) for use in further studies. Supernatants from these hybrid lines have continued to compete in the ABA-binding RIA throughout 4 months of continuous culture.

FACS Analysis. BW5147, F3, and F12 cells from cultures at the mid-logarithmic phase of growth (5–8 × 10^5 cells per ml) were examined for cell surface expression of immunoglobulin, Thy 1.2, and Lyt markers by indirect immunofluorescence, using the monoclonal reagents described in *Materials and Methods*. Fig. 2 *Top* shows that BW5147 cells are negative for immunoglobulin, Thy 1.2, Lyt 1.2, and Lyt 2.2. In contrast, Fig. 2 *Middle* and *Bottom* demonstrates that while F3 and F12 are immunofluorescence negative for surface immunoglobulin and Lyt 2.2 antigen, these hybrid cell lines exhibit the Thy 1.2^+, Lyt 1.2^+ phenotype.

***In Vivo* Activity of the T Cell Hybrid Supernatants.** Supernatants from BW5147, A4, F3, F12, and H11 mid-logarithmic-phase cultures were tested for suppressor activity in the ABA–DTH suppressor system. A/J mice were immunized for DTH with ABA-coupled syngeneic spleen cells and treated with 200 μl of the cell-free culture supernatants administered intravenously for 5 days. The mice were then challenged with ABA and footpad swelling was measured 24 hr later as a gauge of DTH. Fig. 3 shows that BW5147 supernates had no effect on the DTH response and neither did the supernatant from F3 or H11 cultures. In contrast, A4 (data not shown) and F12 supernatants administered for 5 days suppressed the ABA DTH response to the background levels elicited by challenge alone.

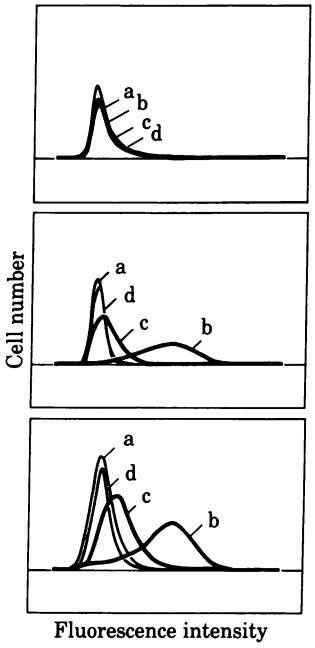

FIG. 2. Cytofluorograph profiles of hybrid cell lines. For each experiment, 5 × 10^4 BW5147 (*Top*), F3 (*Middle*), and F12 (*Bottom*) cells from 3-day cultures were analyzed by FACS II after staining with fluorescein isothiocyanate-derivatized rabbit anti-mouse immunoglobulin alone (curves a) or with anti-Thy 1.2 (curves b), anti-Lyt 1.2 (curves c), or anti-Lyt 2.2 (curves d) antibodies followed by fluorescein isothiocyanate-derivatized rabbit anti-mouse immunoglobulin.

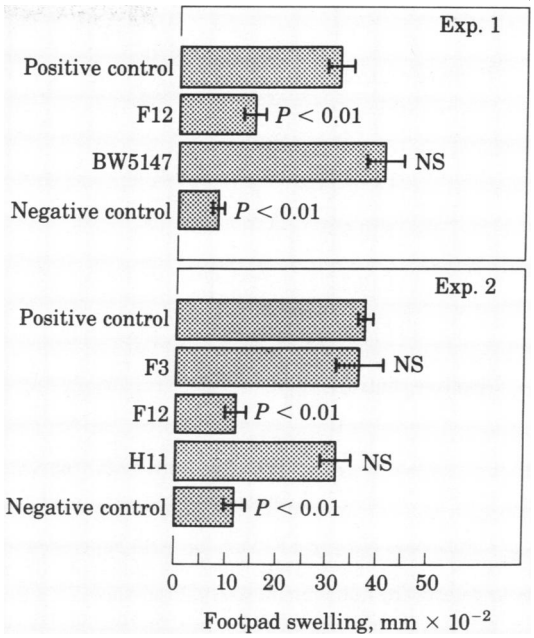

FIG. 3. Hybrid supernatant administered intravenously suppresses the *in vivo* DTH response to ABA. Groups of four A/J mice were immunized with ABA-coupled syngeneic spleen cells and treated intravenously with the indicated culture supernatants for the next 5 days (200 μl/day). The mice were then challenged with ABA in the footpads and swelling was measured 24 hr later. The positive control group was immunized and challenged, while the negative control was only challenged. BW5147 parent lymphoma, F3, and H11 supernatants do not suppress the DTH response, whereas F12 supernatant is highly suppressive ($P < 0.01$). Error bars indicate SEM; NS, not significant.

In successive experiments over 4 months, F3 has remained negative for suppressor activity while F12 has maintained specific suppressor activity for the ABA hapten. To titrate the suppressor activity, culture supernatant was diluted with P_i/NaCl and injected into mice, 0.2 ml per mouse per day. More than 60% suppression was obtained by 1:50 dilution (Fig. 4). When the supernatant was diluted to 1:100, the suppression decreased to 40% but was still statistically significant ($P < 0.05$).

F12 Is a Cloned T Cell Hybrid Line. The clonal nature of the F12 cell line is supported by three lines of evidence. The first is the fact that the initial seeding of the fused cells was at

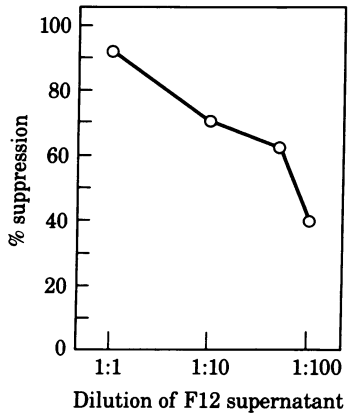

FIG. 4. Titration of ABA suppressive activity in F12 culture supernatants. F12 culture supernatant was diluted as indicated and tested in groups of four A/J mice for suppression of DTH to ABA (see legend to Fig. 3). The F12 supernatant material remained suppressive at a 1:100 dilution ($P < 0.05$).

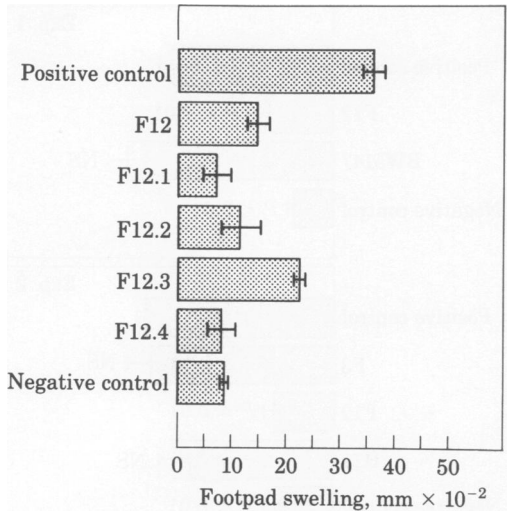

FIG. 5. Subclones of hybrid F12 exhibit ABA suppressor activity. Twenty-four of 24 agar-derived F12 subclones exhibited ABA-binding activity as detected by radioimmunoassay. From these 24, 4 subclones were randomly selected and recoded F12.1–4, and culture supernatants were tested for *in vivo* suppression of ABA DTH (see legend to Fig. 3). All 4 subclones induced significant suppression of the DTH response to ABA that was equivalent to that elicited by the parent line, F12. Error bars indicate SEM.

limiting dilution. Second, the immunofluorescence analysis patterns for Thy 1.2 and Lyt 1.2 are unimodal and therefore indicative of a homogeneous cell population. Finally, F12 cells were recloned on soft agar and 24 subclones were picked for expansion and testing. Twenty-four of 24 F12 agar subclones showed significant inhibition of the ABA RIA. Four randomly selected subclones were tested *in vivo* for ABA DTH suppressor activity (Fig. 5). All four subclones inhibited the DTH response to ABA.

DISCUSSION

Detailed investigations of the function of specific T cell subsets and T cell-derived factors will be greatly enhanced by the use of cloned cell lines or hybrid T cell lines. There have been several recent reports of similarly derived T cell hybrids with antigen-binding (17–20, 27) or *in vitro* antigen-specific suppressor activity (12–16). We describe here the polyethylene glycol-facilitated generation of T cell somatic hybrids from mice possessing suppressor T cells specific for the hapten ABA. From a large number of fusion events we selected four hybrid lines that produce an ABA-binding material as detected by RIA. This assay, in which binding of a monoclonal anti-ABA antibody to ABA-BSA-coated plates is inhibited by "factor," is designed to detect only non-immunoglobulin ABA-binding molecules. Of the four lines producing ABA-binding factors, A4 and F12 were shown to be active in an *in vivo* assay for suppression of DTH to ABA. Supernatants from the two nonsuppressive ABA-binding lines were not assayed for other functions. It is possible that the "nonsuppressor" hybrids may be helper, cytotoxic, or proliferating cells with ABA specificity. Pacifico and Capra (20) have reported an ABA-specific T cell hybrid that produces an ABA-binding material assayed in a similar competitive RIA. This cell line was not lytic in a polyspecific cytotoxicity assay, and no function has as yet been reported for this hybrid. Determination of whether such antigen-binding materials (F3, Pacifico–Capra) are defective—i.e., lack an "effector" component—or have an as yet unrevealed immune function may yield information on the structure–function characteristics of immune T cell-derived factors.

Of the four T cell hybrid lines selected by RIA, the line designated F12 has been extensively studied. The hybrid nature of this line is documented by its initial selection in HAT medium and the demonstration on the F12 surface Thy 1 antigens normally restricted to the lymphoid parent genotype. FACS analysis of F12 with anti-Lyt antisera showed that the cell line is Lyt $1^+,2^-$. The unimodal fluorescence patterns and the fact that the cells were plated after fusion at limiting dilution suggest that F12 is a cloned cell line. In a test of the clonality of F12, the line was plated on soft agar and 24 subclones were chosen for analysis. The 24 subclones were tested in the ABA-binding assay and all were found to produce ABA-binding supernatant factors. From the 24 subclones of F12, 4 were randomly chosen and tested for suppression of ABA DTH *in vivo;* all 4 subclones produced significant levels of suppression equivalent to the level produced by the parent F12 clone.

The F12 suppressor factor appears to provide a potent inhibitory signal for idiotypic antigen-specific suppression of precytolytic T cell generation (unpublished data) and DTH. This suggests that the F12 hybrid T cell line is representative of the inducer suppressor or Ts_1 class of regulatory T cells, consistent with the Thy 1^+, Lyt $1^+,2^-$, Ig^- phenotype described. However, CRI determinants and $I\text{-}J^k$-encoded structures, surface markers of conventional Ts_1 (2), could not be detected on the *in vitro*-maintained F12 cell line by indirect immunofluorescence techniques, FACS, or complement-dependent cytotoxicity. In contrast, the secreted soluble suppressor products obtained from culture supernatants of this hybrid T cell line bear both CRI and *I-J* subregion-encoded determinants. In parallel, recent experiments utilizing F12 cells maintained as ascites in syngeneic mice have revealed that such *in vivo*-grown F12 cells express cell surface CRI as detected by immunofluorescence. This growth-condition dependence of cell surface CRI expression by F12 can be exploited to identify the signals that modulate the surface antigens of this antigen-specific hybrid T cell line.

The authors thank Ed Luther for the FACS analysis and Nancy Axelrod and Teresa Greenberg for patient preparation of the manuscript. This work was supported by Grant AI-14732-04 and Training Grant 5T32-CA-09103-05 from the National Institutes of Health.

1. Bach, B. A., Sherman, L., Benacerraf, B. & Greene, M. I. (1978) *J. Immunol.* **121,** 1460–1468.
2. Sy, M.-S., Dietz, M. H., Germain, R. N., Benacerraf, B. & Greene, M. I. (1980) *J. Exp. Med.* **151,** 1183–1195.
3. Weinberger, J. Z., Germain, R. N., Benacerraf, B. & Dorf, M. E. (1980) *J. Exp. Med.* **152,** 161–169.
4. Tada, T., Taniguchi, M. & Takemori, T. (1975) *Transplant. Rev.* **26,** 106–129.
5. Pierce, C. W. & Kapp, J. A. (1976) *Contemp. Top. Immunobiol.* **5,** 91–143.
6. Germain, R. N. & Benacerraf, B. (1980) *Springer Sem. Immunopathol.* **3,** 93–127.
7. Hellström, K. E., Hellström, I. & Nepom, J. T. (1977) *Biochim. Biophys. Acta* **473,** 121–148.
8. Greene, M. I., Fujimoto, S. & Sehon, A. (1977) *J. Immunol.* **119,** 757–764.
9. Perry, L. L., Benacerraf, B. & Greene, M. I. (1978) *J. Immunol.* **121,** 2144–2147.
10. Dietz, M. H., Sy, M.-S., Greene, M. I., Nisonoff, A., Benacerraf, B. & Germain, R. N. (1980) *J. Immunol.* **125,** 2374–2379.
11. Dietz, M. H., Sy, M.-S., Benacerraf, B., Nisonoff, A., Greene, M. I. & Germain, R. N. (1981) *J. Exp. Med.* **153,** 450–463.
12. Taniguchi, M. & Miller, J. F. A. P. (1978) *J. Exp. Med.* **148,** 373–382.
13. Watanabe, T., Kimoto, M., Maruyama, S., Kishimoto, T. & Yamamura, Y. (1978) *J. Immunol.* **121,** 2113–2117.
14. Taussig, M. J., Corvalan, J. R. F., Binns, R. M. & Holliman, A. (1979) *Nature (London)* **277,** 305–308.

15. Kapp, J. A., Araneo, B. A. & Clevinger, B. C. (1980) *J. Exp. Med.* **152**, 235–240.
16. Nelson, K., Cory, J., Hellström, I. & Hellström, K. E. (1980) *Proc. Nat. Acad. Sci. USA* **77**, 2866–2870.
17. Whitaker, R. B. & Ruddle, N. H. (1980) *Cell. Immunol.* **55**, 56–65.
18. Ruddle, N. H., Beezley, B., Lewis, G. K. & Goodman, J. W. (1980) *Mol. Immunol.* **17**, 925–932.
19. Goodman, J. W., Lewis, G. K., Primi, D., Hornbeck, P. & Ruddle, N. H. (1980) *Mol. Immunol.* **17**, 933–941.
20. Pacifico, A. & Capra, J. D. (1980) *J. Exp. Med.* **152**, 1289–1301.
21. Galfre, G., Howe, S. C., Milstein, C., Butcher, C. W. & Howard, J. C. (1977) *Nature (London)* **266**, 550–555.
22. Ruddle, N. H. (1978) *Curr. Top. Microbiol. Immunol.* **81**, 203–211.
23. Littlefield, J. W. (1964) *Science* **145**, 709.
24. Lamoyi, E., Estess, P., Capra, J. D. & Nisonoff, A. (1980) *J. Immunol.* **124**, 2834–2840.
25. Nepom, J. T., Benacerraf, B. & Germain, R. N. (1981) *J. Immunol.*, in press.
26. McConahey, P. J. & Dixon, F. J. (1966) *Int. Arch. Allergy Appl. Immunol.* **29**, 185–192.
27. Ruddle, N. H., Beezley, B. B. & Eardley, D. D. (1980) *Cell. Immunol.* **55**, 42–55.