MONOCLONAL SUPPRESSOR FACTOR SPECIFIC FOR LACTATE DEHYDROGENASE B

I. Mechanism of Interaction Between the Factor and Its Target Cells*

BY ZENRO IKEZAWA, CONSTANTIN N. BAXEVANIS, MAKOTO NONAKA, RYO ABE, TOMIO TADA, ZOLTAN A. NAGY, AND JAN KLEIN

From the Max-Planck-lnstitut für Biologie, Abteilung Immunogenetik, 7400 Tübingen, Federal Republic of Germany, and the Department of Immunology, Faculty of Medicine, University of Tokyo, Tokyo 113, Japan

Antigen-specific, T cell-derived factors have been the subject of extensive studies as possible mediators of immunoregulatory interactions (reviewed in references 1–3). Functionally, two kinds of factor, helper and suppressor, can be distinguished. The suppressor factors (TsF), again, fall into two categories, namely, inducer factors (TsiF) that play a role in communication between T cell sets of the suppressor pathway, and effector factors (TseF) that cause ultimate suppression. Because the immune response to a single antigen may involve a multitude of regulatory factors, it is mandatory to establish a pure source of these mediators for further biological and biochemical studies. To this end, several laboratories have produced T cell lines or T cell hybridomas that secrete monoclonal TsF. While the role of some of these TsF in the suppressor pathway has not yet been determined (4–6), other factors have been shown to possess TsiF (7, 8) or TseF (9–13) activity. Thus, there exists now a battery of monoclonal TsF suitable to study cellular interactions in different suppressor pathways. However, there are other obstacles to these studies, namely, that the cells participating in most known suppressor pathways have not been identified, and the effector functions used as a readout of suppression themselves involve complex cellular interactions (4–6, 9–14).

We have characterized recently a suppressor pathway that regulates the immune response to lactate dehydrogenase B (LDHB) (15–17). The suppressor effector (Tse) cell in this system is an Lyt-1+2+ cell that becomes activated by the recognition of LDHB together with Eα (Eα,Eβ) molecules of antigen-presenting cells (APC) and by an additional, nonspecific, Tsi cell-derived signal. The target of the Tse cell is a proliferating Lyt-1+2− cell that recognizes LDHB together with the A (Aβ,Aβ) molecules of the APC. The latter cells probably include the LDHB-specific T helper (Th) cells, since the strain distribution of responsiveness to LDHB is identical in terms of both T
cell proliferation and antibody production (15, 18). The interaction between Tse and Th is antigen-specific and major histocompatibility complex (Mhc)-restricted, and the suppression is manifested in the inhibition of the LDH\textsubscript{B}-specific, A-restricted proliferation of the Th cells. Because it is so well characterized, the anti-LDH\textsubscript{B} response provides an ideal system to study the mechanism of Tse–Th cell interaction.

We report here the functional characterization of monoclonal TseF secreted constitutively by T cell hybridomas. The latter were produced by fusing a long-term, LDH\textsubscript{B}-specific Tse cell line with the BW5147 thymoma. We use this factor to clarify the mechanism of Tse–Th cell interaction.

**Materials and Methods**

*Mice.* 8–15-wk-old male and female mice were obtained from our colony at the Max Planck Institute for Biology. The strains and their alleles at \(H-2\) loci are listed in Tables IV and VI.

*Antigens and Immunization.* LDH\textsubscript{B} (LDH-H4 from pig heart; Boehringer, Mannheim, F.R.G.), keyhole limpet hemocyanin (KLH; Calbiochem, Giessen, F.R.G.), poly(glu\textsubscript{50}ala\textsubscript{50}tyr\textsubscript{20}) (GAT; Miles-Yeda, Rehovoth, Israel) and UPC 10 (purified IgG\textsubscript{m} myeloma protein; Bionetics, Fresenius, Stuttgart, F.R.G.) were emulsified at 1/1 (vol/vol) ratio with complete Freund's adjuvant (CFA; Difco Hedinger, Stuttgart, F.R.G.), and 0.05 ml of the emulsion, containing 0.05 mg of antigen, was injected into mice subcutaneously, at the tail base. Concanavalin A (Con A) was purchased from Deutsche Wellcome (Burgwedel, F.R.G.).

*Monoclonal Antibodies.* Monoclonal A- and E-specific antibodies secreted by hybridomas B15-124R1 (anti-Ia.m2), 13/4 (anti-Ia.m7; reference 19; the cell lines were obtained from Dr. G. J. Hämmerling, German Cancer Research Center, Heidelberg, F.R.G.), 10.2.16 (anti-Ia.m17; reference 20; obtained from the Salk Institute, San Diego, CA), Y17 (anti-Ia.m44; reference 24; a gift from Dr. D. B. Murphy, Yale University School of Medicine Dept. of Pathology, New Haven, CT), and 25-9-17 (reacts with an unassigned A\textsuperscript{b} determinant; reference 22; the hybridoma was obtained from Dr. D. H. Sachs, Transplantation Biology Section, Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD) were used in ascites form. Monoclonal antibodies 1L9, 2L2, and 14P, specific for a molecule controlled by a locus in the A\textsubscript{b}-E\textsubscript{b} interval and selectively expressed by T cells, were produced as described previously (23).

*Production of Hybridomas Secreting LDH\textsubscript{B}-Specific TseF.* Long-term Tse cell line was established from LDH\textsubscript{B}-immune lymph node-cells of B10.A(2R) (suppressed nonresponder) mice. The Lyt-1\textsuperscript{+}2\textsuperscript{+} set containing LDH\textsubscript{B}-specific Tse was separated by positive selection of Lyt-1\textsuperscript{+}2\textsuperscript{+} and Lyt-1\textsuperscript{−}2\textsuperscript{+} cells with a monoclonal Lyt-2.2 specific antibody followed by the selection of Lyt-1\textsuperscript{+}2\textsuperscript{+} cells with a monoclonal Lyt-1.2 specific antibody (17), and the cells (1 × 10\textsuperscript{5}/ml) were cultured in Alpha minimal essential medium (Gibco, BCK Biocuh-Chemie, Karlsruhe, F.R.G.) supplemented with 10% FCS, antibiotics, \(\gamma\)-glutamine, and 2-mercaptoethanol, in the presence of 15 \(\mu\)g/ml LDH\textsubscript{B} and syngeneic, irradiated (3,300 R) spleen cells (2.5 × 10\textsuperscript{5}/ml) as feeder cells, for 1 wk, followed by another week of culture in the presence of antigen, feeder cells, and T cell growth factor (TCGF; 48 h supernate of Con A-stimulated mouse spleen cells). After three such culture cycles, the cells were expanded by weekly restimulations with LDH\textsubscript{B}, TCGF, and feeder cells. The cultured cells were found to suppress the LDH\textsubscript{B}-specific proliferation of Lyt-1\textsuperscript{+}2\textsuperscript{+} cells in an antigen-specific and Mhc-restricted fashion. Cells of the 4-mo-old Tse line were fused with the BW5147 thymoma, and the hybridomas were selected using standard methods (4, 24).

The hybridoma supernates were screened for suppression of the antigen-specific proliferation of LDH\textsubscript{B}-primed syngeneic Lyt-1\textsuperscript{+}2\textsuperscript{+} cells. Six hybridomas (2RL-Ts-1 through 6) were found to secrete, in the absence of antigen, a suppressive material. Two of these hybridomas (2RL-Ts-1 and 5) were recloned (at a density of 0.3 cell/well). All clones tested were positive, and secreted TseF that was functionally indistinguishable from that produced by uncloned lines. Therefore, in the experiments described here, supernates of cloned and uncloned hybridomas were used indiscriminately.

*Testing the Effect of TseF on Antigen-specific T Cell Proliferation.* Immune lymph-node cells or the Lyt-1\textsuperscript{+}2\textsuperscript{−} fraction thereof (obtained by separation on anti-mouse IgG-coated dishes after
Antigen-Specificity of TseF Produced by Hybridoma Cells

| Source of supernatant* | Anti-LDH\textsubscript{B} response\textsuperscript{a} |
|------------------------|---------------------------------|
|                        | (B10.A(2R), Lyt-\textsuperscript{1+2}) | Anti-LDH\textsubscript{B} response\textsuperscript{a} |
|                        | (B10.A(2R), Lyt-\textsuperscript{1+2}) | Anti-KLH response\textsuperscript{a} |
|                        | (B10.A(2R), unseparated) | Anti-GAT response\textsuperscript{a} |
|                        | (B10.A(2R), unseparated) | |
| BW5147                 | 80,669 20.0 | 55,338 13.8 | 14,002 6.2 | 8,521 4.3 |
| 2RL-Ts-1               | 936 1.2 | 567 1.1 | 15,278 6.6 | 9,034 4.7 |
| 2RL-Ts-2               | 933 1.2 | 3,735 1.3 | 20,651 6.5 | 12,025 4.0 |
| 2RL-Ts-3               | 526 1.1 | 3,722 1.2 | 20,172 6.4 | 10,676 4.1 |
| 2RL-Ts-4               | 268 1.0 | 1,498 1.1 | 20,319 5.4 | 12,994 4.5 |
| 2RL-Ts-5               | 1,134 1.4 | 457 1.0 | 19,036 6.6 | 11,050 5.0 |
| 2RL-Ts-6               | 2,506 1.0 | 1,987 1.1 | 21,045 5.2 | 11,934 3.8 |

* Hybridoma supernates were added to cultures at a 1:4 final dilution.

1 Proliferation of $4 \times 10^6$ primed T cells in the presence of 15 \(\mu\)g/ml of LDHB, 10 \(\mu\)g/ml of KLH, or 50 \(\mu\)g/ml of GAT.

Results

The Effect of Hybridoma-produced TseF on Th Cells is Antigen-specific and Mhc-Restricted. Hybridoma supernates, when included in the 3-d proliferation assay, inhibited to background levels the response of LDHB-primed Lyt-\textsuperscript{1+2} cells from B10.A(2R) mice (unseparated cells from these strains are nonresponders to LDHB because of the presence of Lyt-\textsuperscript{1+2} cells; references 15-17), but did not influence the proliferative response of KLH-primed and GAT-primed B10.A(2R) cells (Table I). Thus the suppressive effect of these factors is antigen-specific. However, the LDHB-specific TseF completely suppressed the proliferative response of T cells to the

\textsuperscript{2} Baschal, C. N., Z. A. Nay, and J. Klein. 1983. The nature of the interaction between suppressor and helper T cells in the response to LDHB. Manuscript submitted for publication.
**TABLE II**

Cross-Suppression of IgG₂a-Specific T-cell Proliferation by the LDH₈-Specific TseF

| Source of supernatant* | Proliferative response of Th cells (Lyt-1⁺2⁻) from B10.A (4R) mice primed to UPC 10 | Anti-UPC 10 response | Anti-LDH₈ response |
|------------------------|----------------------------------------------------------------------------------------|----------------------|-------------------|
|                        |                                                                                       | Δ cpm                | S.I.              | Δ cpm | S.I. |
|                        |                                                                                       |                     |                   |       |      |
| -                      |                                                                                       | 330117              | 117.2             | 1320  | 1.5  |
| BW5147                 |                                                                                       | 308972              | 43.1              | ND    |      |
| 2RL-Ts-1               |                                                                                       | 2287                | 1.3               | ND    |      |

* See footnotes to Table I.
† Proliferation of 4 × 10⁵ T cells in the presence of 125 μg/ml of UPC 10 or 15 μg/ml of LDH₈.
ND, not done.

**TABLE III**

Time Course of Action of TseF on Th Cells

| Preincubation of Th cells with TseF | Proliferative response of LDH₈-prime d Th cells (Lyt-1⁺2⁻) after contact with 2RL-TsF | Proliferative response of GAT-prime d Th cells (Lyt-1⁺2⁻) after contact with 2RL-TsF | Anti-LDH₈ response | Con A response | Anti-GAT response | Con A response |
|-----------------------------------|------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------|-------------------|----------------|-------------------|----------------|
|                                   |                                                                                         |                                                                                 | Δ cpm             | S.I.           | Δ cpm             | S.I.           |
| 0                                 | 78,851 (25.1)                                                                            | 126,195 (68.6)                                                               | 15,748 (7.4)      | 138,986 (57.7) |
| 30 min                            | 80,476 (39.3)                                                                            | 113,528 (55.1)                                                              | 17,067 (6.6)      | 107,438 (36.2)  |
| 1 h                               | 92,667 (35.0)                                                                            | 114,597 (43.1)                                                              | 15,566 (6.5)      | 110,571 (40.2)  |
| 2 h                               | 76,531 (34.0)                                                                            | 110,592 (46.7)                                                              | 18,171 (8.3)      | 103,527 (42.2)  |
| 4 h                               | 125 (1.1)                                                                                | 105,389 (60.7)                                                              | 15,003 (9.8)      | 102,832 (61.1)  |
| 8 h                               | -235 (0.9)                                                                               | 107,019 (70.6)                                                              | 16,142 (8.8)      | 103,711 (51.2)  |

* Cells were incubated at 37°C with hybridoma supernate at a 1:2 dilution, washed, and tested for proliferation.
† The concentration of Con A was 25 μg/ml; LDH₈ and GAT were used at concentrations given in Table I.

IgG₂a myeloma protein UPC 10 (Table II). This finding confirms our previous observation that the Tse cells involved in the anti-LDH₈ and anti-IgG₂a responses cross-react completely (28). The crude hybridoma supernates caused complete suppression of T cell proliferation at a dilution up to more than 1:500 (data not shown). The data in Table III demonstrate that a short term (4 h) exposure of cells to the factor is sufficient to cause complete suppression, indicating that the factor is a TseF that acts directly on the Th cells. The interaction of TseF with Th is Mhc-restricted (Table IV). The strains used as Th donors map this restriction to the A₁⁻E₈ interval, and excludes the involvement of all other H-2 regions (also the J region), and of non-H-2 genes. Since the action of the LDH₈-specific Tse cells on Th cells is also short-term, antigen-specific, and A-restricted, the hybridoma TseF can be considered as the secreted form of the molecule responsible for the Tse-Th cell interaction.

Mhc-Restricted Absorption of TseF Activity with LDH₈-Specific Th Cells. The suppres-
### Table IV

**H-2 Mapping of TseF Restriction**

| Strain        | Alleles at H-2 regions | Proliferative response of Th cells (Lyt-1+2-) treated with:* |
|---------------|------------------------|---------------------------------------------------------------|
|               |                        | BW5147 | 2RL-Ts-1 | 2RL-Ts-2 | 2RL-Ts-5 |
|               |                        | Δ cpm  | (S.I.)   | Δ cpm  | (S.I.)   | Δ cpm  | (S.I.)   |
| B10.A(2R)     | k k k k b              | 155,412 (15.1) | -1 (1.0) | -2,163 (0.9) | 1,471 (1.1) |
| B10.A(4R)     | k k k k b              | 130,368 (48.2) | -32 (1.0) | 802 (1.2) | 568 (1.1) |
| B10.A(5R)     | b b k k d              | 123,249 (39.6) | 115,453 (28.9) | 127,165 (19.9) | 110,223 (24.6) |
| B10.A(3R)     | b b b k d              | 139,033 (12.5) | 138,469 (12.6) | ND | 137,574 (10.7) |
| B10.S(9R)     | b b b k d              | 142,147 (23.4) | 121,319 (19.7) | ND | 116,946 (14.8) |
| B10.MBR       | b k k k q              | 134,185 (61.4) | -91 (1.0) | 421 (1.1) | 381 (1.1) |
| B10.AQR       | q k k k b              | 122,624 (17.7) | -718 (0.9) | ND | 696 (1.1) |
| C57BL/6       | b b b b b              | 131,065 (28.7) | 133,404 (26.5) | 146,938 (16.3) | 156,367 (22.2) |
| CBA           | k k k k k              | 303,645 (44.8) | 2,535 (1.4) | 2,809 (1.3) | 1,494 (1.2) |

* The TseF was added to cultures at a final dilution of 1:20.

**Table V**

**Absorption of TseF with Antigen-Specific Th Cells**

| Source of supernatant | Absorbing cells* | Proliferative response of Th (Lyt-1+2-) cells to LDHb |
|-----------------------|-------------------|-----------------------------------------------------|
|                       |                   | Strain  | Δ cpm  | S.I.   |
| BW5147                | None              | B10.AL  | 59,792 | 29.2   |
| 2RL-Ts-5              | None              | B10.AL  | 329    | 1.1    |
| 2RL-Ts-5              | LDHB-Primed syngeneic Lyt-1+2" | B10.AL  | 57,581 | 22.1   |
| 2RL-Ts-5              | GAT-Primed syngeneic Lyt-1+2" | B10.AL  | 103    | 1.0    |
| BW5147                | None              | B10.TL  | 166,071 | 14.3  |
| 2RL-Ts-1              | None              | B10.TL  | -59    | 1.0    |
| 2RL-Ts-1              | LDHB-Primed syngeneic Lyt-1+2" | B10.TL  | 150,583 | 12.5  |
| 2RL-Ts-1              | LDHB-Primed syngeneic Lyt-1+2", Lyt-1+2" | B10.TL  | 511    | 1.0    |
| BW5147                | None              | B10.AQR | 115,704 | 17.7  |
| 2RL-Ts-5              | None              | B10.AQR | 698    | 1.1    |
| 2RL-Ts-5              | Unprimed B10.A(2R) spleen cells | B10.AQR | -962   | 0.9    |
| 2RL-Ts-5              | LDHB-Pulsed B10.A(2R) spleen cells | B10.AQR | 74     | 1.0    |
| 2RL-Ts-5              | Unprimed B10.A(4R) spleen cells | B10.AQR | 767    | 1.1    |
| 2RL-Ts-5              | LDHB-Pulsed B10.A(4R) spleen cells | B10.AQR | 205    | 1.0    |

* 1 ml of TseF diluted 1:4 or 1:10 was absorbed with 4 × 10^7 and 2 × 10^7 cells, respectively.

The TseF activity of hybridoma supernates can be removed completely by absorption with LDHB-primed syngeneic [B10.A(2R)] Lyt-1+2 cells (Table V). In contrast, syngeneic Lyt-2-bearing cells primed to LDHB, or syngeneic Lyt-1+2" cells primed to GAT do not absorb the factor. Similarly, LDHB-pulsed or unpulsed spleen cells from unprimed syngeneic mice are unable to absorb the factor. Thus the TseF binds to the cells on which it acts (LDHB-specific Th cells), but fails to bind to LDHB-primed non-Th cells, to Th cells of irrelevant specificity, or to APC. The binding of TseF to Th cells is subject to the same genetic restriction as its functional activity, that is, the factor is only absorbed by LDHB-primed Th cells that share genes in the $A_e-A_B$ interval with...
Table VI

| Source of TseF | Absorbing LDHB-primed Lyt-1+2- cells* | Proliferative response of Th (Lyt-1+2-) cells |
|---------------|----------------------------------------|---------------------------------------------|
|               | Strain | Alleles at H-2 regions | Strain | Δ cpm | S.I. |
| 2RL-Ts-1      | A/JED | k k k k b | B10.A(2R) | -314 | 1.0 |
| 2RL-Ts-1      | 2RL-Ts-1 | B10.A(4R) | k k b b b | B10.A(2R) | 52,166 | 5.2 |
| 2RL-Ts-1      | B10.A(5R) | b b k k d | B10.A(2R) | 722 | 1.1 |
| 2RL-Ts-1      | C57BL/6 | b b b b | B10.AQR | 945 | 1.1 |
| 2RL-Ts-1      | CBA | k k k k k | B10.A(2R) | 51,589 | 4.5 |
| 2RL-Ts-5      | -- | -- | B10.AQR | 945 | 1.1 |
| 2RL-Ts-5      | A.TFR1 | s k k j | B10.AQR | 106,074 | 11.5 |
| 2RL-Ts-5      | B10.S(9R) | s s k k d | B10.AQR | 457 | 1.1 |

* 1 ml of 1:4 diluted TseF was absorbed with 2 X 10^7 cells.

The factor-producing cells (Table VI). Thus the factor acts after binding to its target cell in an antigen-specific and Mhc-restricted fashion.

Antigen Requirement of TseF-Th Cell Interaction. The antigen specificity of TseF-binding to Th cells can reflect either a direct receptor–receptor interaction (for example, idiotype–antiidiotype reaction) or an indirect interaction through an antigen bridge. To distinguish between these possibilities, we tested whether antigen is necessary for the TseF to act on Th cells. To conclusively demonstrate this point, one first has to ensure that the experimental system is devoid completely of antigen. This requirement is met in the case of the TseF used, since the factor is secreted by hybridomas in the absence of LDHB. However, the Th cells might have carried LDHB, and so, to preclude this possibility we trypsinized them to remove the surface-bound antigen. Because trypsin also attacks cell-membrane proteins (29), the cells were allowed to resynthesize their receptors during an overnight incubation in antigen-free medium. The interaction of these cells with TseF was then tested in a short term (6 h) culture, with or without LDHB. The cells were then washed, and tested for antigen-specific proliferation. Suppression ensued during the 6-h exposure of Th cells to TseF only when antigen was added to the system (Table VII). Furthermore, trypsin-treated Th cells regenerated their capacity to proliferate to LDHB but they were unable to absorb TseF in the absence of antigen (Z. Ikezawa, unpublished results). We conclude, therefore, that an antigen-bridge is necessary for the TseF to bind to and act on Th cells.

The Mechanism of Mhc-Restriction in the Interaction between TseF and Th Cells. The data in Tables IV and VI have demonstrated that the TseF only suppresses Th cells that share the A^aE^b interval genes with the factor-producing cells. We have previously shown that the same genetic restriction applies to the Tse-Th cell interaction in the anti-LDHB response (17). Further analysis has revealed that the Tse-Th cell interaction is determined by the receptor of Th cells (anti-LDHB + A^a) and the presence of A^a-alleles in the genome of Tse cells. We inferred from these data that the interaction is based on the recognition by Th cells of A^a-controlled determinants expressed on the
TABLE VII

Antigen Requirement in the TseF-Th Cell Interaction: LDH_B-Primed B10.A(4R) Th (Lyt-1^+2^-) Cells after Trypsin Treatment and Overnight Incubation

| Short-term (6 h) preincubation with LDH_B | Proliferative response to LDH_B |
|------------------------------------------|--------------------------------|
| Supernatant* | LDH_B | Δ cpm | S.I. |
| BW5147 sup | - | 158,776 | 25.1 |
| BW5147 sup | + | 146,072 | 24.0 |
| 2RL-TsF-1 | - | 162,980 | 24.1 |
| 2RL-TsF-1 | + | 2,293 | 1.3 |

* Cells were preincubated at 37°C with hybridoma supernates at a 1:4 dilution, washed and tested for proliferation.

Table VIII

Inhibition of TseF Activity by T Cell-specific Anti-A_k Antibodies

| Pretreatment of factors with antibodies (final dilution) | Proliferative response of B10.A(4R) Th cells (Lyt-1^+2^-) after 6 h incubation with BW5147 sup.* 2RL-TsF-5* |
|----------------------------------------------------------|-----------------------------------------------------|
|                                                         | Δ cpm (S.I.) | Δ cpm (S.I.) |
| 10.2.16:aElam.17 (1:200)                                  | 296,704 (89.1) | 190 (1.0) |
| 15.124.4:aElam.2 (1:200)                                  | 148,377 (54.2) | 189 (1.1) |
| 25.9.17:aAb (1:200)                                       | 189,470 (56.1) | 45 (1.0) |
| 13.4:aElam.7 (1:1,000)                                    | 237,411 (61.4) | 664 (1.0) |
| Y17:aElam.44 (1:1,000)                                    | 225,713 (64.0) | 345 (1.1) |
| 1L9:aAk-T (1:1,000)                                       | 218,754 (73.0) | 646 (1.2) |
| 14P:aAk-T (1:1,000)                                       | 222,535 (73.5) | 167,435 (53.6) |
| IL9:aAk-T (1:800)                                         | 230,138 (69.9) | 246,514 (68.4) |

* Hybridoma supernates were used at a 1:10 final dilution.

Tse cells. If this hypothesis is valid, the TseF that exhibits identical genetic restriction to that of the Tse cell, must carry A^k-controlled antigenic determinants. We therefore tested whether A^k-specific monoclonal antibodies can abrogate the effect of TseF on Th cells. The factor was preincubated with the antibodies and then allowed to interact with LDH_B-primed Lyt-1^+2^- cells for 6 h. The cells were then washed and tested for LDH_B-specific proliferation in a 3-d assay. As shown in Table VIII, antibodies against the known class II Mhc molecules (A and E) did not interfere with the activity of TseF. Interestingly, however, monoclonal antibodies IL9 and 14P that recognize A^k-A^k interval-controlled T-cell specific determinants (23) completely neutralized the TseF. Another antibody of the T-cell-specific "anti-A^k" series, 2L2, had the same effect, whereas antibody 1G6 that reacts with Tsi cells (T. Tada et al., unpublished data) had no effect on the TseF (Z. Ikezawa, unpublished results). Antibodies 1L9, 2L2, and 14P, when included in the assay cultures, neutralized the added TseF, but did not block the A^k-A^k-restricted proliferation of Th cells (Table IX). Furthermore, the antibodies did not affect the capacity of Th cells to absorb the TseF (Z. Ikezawa, unpublished data). Thus, the antibodies recognize A^k-like determinants carried by the factor, but absent from the APC and Th cells. We have investigated also whether
the interaction of A-like determinants of the TseF with Th cells depends on the Mhc haplotype or the receptor specificity of the Th cells. We generated in vitro CBA (H-2k) and B10.S (H-2d) Th cells that recognize LDHB in a self-restricted manner (A-like and A-like-restricted, respectively), or in the context of allogeneic A molecules (CBA anti-LDHB + A, and B10.S anti-LDHB + A). As shown in Table X, the Th cells, irrespective of their Mhc haplotype, are suppressed by the factor, provided that they recognize LDHB together with A-like molecules. The interaction of A-like determinants of TseF with the anti-A receptors of Th cells is abrogated by the T-cell specific "anti-A-like" antibodies 1L9 and 14P. Thus, a functionally important part of the factor is an A-like moiety, which binds to the receptor site imposing A-like restriction on the recognition of LDHB by Th cells.
Discussion

In this communication we have functionally characterized a monoclonal TseF secreted constitutively by hybridomas that were made by fusing a long-term, LDH-B-specific Tse cell line with the BW5147 thymoma. In terms of specificity and function, the TseF is equivalent to the Tse cell itself (17), and can thus be considered the effector molecule of the latter. The factor consists of two functional parts, one that binds LDH-B, and another that carries or mimics the A-like determinant(s) recognized by the Th cells on the APC. For the suppression to occur, a double bond between TseF and Th cell has to be established, that is, the TseF must bind to the anti-LDH-B receptor of Th cells through an antigen bridge, and the A-like determinants of the factor must be bound by the anti-A-like receptor of the Th cell. (Whether the anti-LDH-B and anti-A-like binding sites of Th cells are physically linked remains unknown.) When one of these binding events is prevented from occurring by either the removal of LDH-B from the system, or blocking of the A-like moiety with antibodies, no suppression occurs. The crucial role of A-like determinants in suppression has also been demonstrated by an independent line of evidence, namely by the observation that LDH-B-primed T cells from suppressed nonresponder strains can be turned responder by including in the cultures any of the T-cell-specific monoclonal "anti-A-like" antibodies (23) used in this study (Z. Ikezawa et al., manuscript in preparation). The requirement for a dual recognition explains the antigen-specificity and the A-like restriction of the interaction between the TseF (or the Tse cells) and the Th cells (17). Once the TseF is bound to the Th cells, the suppression ensues within a matter of hours.

Many of the suppressor factors characterized so far resemble the TseF described here, in that they possess a binding site specific for either the immunizing antigen (4-7, 9-13, 30, 31) or a receptor-idiotype of the target cell (8), and carry Mhc-controlled antigenic determinants (4-10, 12, 30, 31). The anti-antigen- or antiidiotype-binding site determines the factor-target cell interaction in all known suppressor systems. The Mhc determinants, in many but not all systems, impose a genetic restriction on the factor-target cell interaction (4, 6-8, 12, 32). Thus, the interaction of these and some additional factors (33, 34) with their targets appears to involve dual recognition, similar to the LDH-B-specific TseF. One important difference between the LDH-B-specific TseF and all other known factors is that the former carries an A-E interval-controlled moiety instead of determinants controlled by the so-called J region. This fortunate situation permitted us to analyze the A-like moiety by comparison with its well-characterized counterpart, the A-E class II molecule. The data have demonstrated that there are determinants shared between APC-derived and TseF-derived A molecules, namely those that serve as restriction elements for antigen-recognition by Th cells. The factor-derived A-moiety carries additional determinants that are absent from APC (or B cell)-derived A molecules (those recognized by the T cell-specific monoclonal "anti-A-like" antibodies), and lacks serologically detectable determinants of the classical A molecule. Whether the two types of A molecule are controlled by different genes remains to be seen. Considering the relative paucity of class II genes in the A-E interval (35), it is more likely that they represent different variants of a single gene product. By analogy, it is tempting to speculate that the so-called J molecule may be an analogous modification of another known class II gene product, perhaps the E chain.
Summary

Hybridomas secreting a monoclonal T suppressor-effector factor (TseF) were produced by fusion of a lactate dehydrogenase B (LDHB)-specific long-term T suppressor-effector (Tse) cell line with the BW5147 thymoma. A short exposure (4 h) to TseF completely suppresses the antigen-specific and A-restricted proliferation of LDHB-primed Lyt-1+2- [possibly helper (Th)] cells. The action of TseF on Th cells, as that of the Tse cells themselves, is antigen-specific and A-restricted. The interaction of TseF with Th cells involves two binding events, of which one occurs via antigen bridge, and the other represents the recognition of a factor-derived Aα-like moiety by the anti-Aα receptor of Th cells. The Aα-like moiety of the TseF carries the determinants that serve as restriction elements for antigen recognition by Th cells, and additional determinants demonstrable by T cell-specific monoclonal "anti-Aα" antibodies, however, it lacks serologically detectable determinants of the B cell-derived AαAβ class II Mhc molecules.

We thank Drs. G. J. Hämmerling, D. B. Murphy, and D. H. Sachs for hybridomas, and Ms. Martha Kimmerle and Ms. Karina Masur for secretarial help.

Received for publication 18 January 1983.

References

1. Tada, T., and K. Okumura. 1979. The role of antigen-specific T cell factors in the immune response. Adv. Immunol. 28:1.
2. Germain, R. N., and B. Benacerraf. 1980. Helper and suppressor T cell factors. Springer Sem. Immunopathol. 3:93.
3. Germain, R. N., and B. Benacerraf. 1981. A single major pathway of T-lymphocyte interactions in antigen-specific immune suppression. Stand. J. Immunol. 13:1.
4. Taniguchi, M., T. Saito, and T. Tada. 1979. Antigen-specific suppressive factor produced by a transplantable I-J-bearing T-cell hybridoma. Nature (Lond.). 278:555.
5. Kapp, J. A., B. A. Aranuco, and B. L. Clevinger. 1980. Suppression of antibody and T cell proliferative responses to l-glutamic acid3L-alanine8L-tyrosine8 by a specific monoclonal T cell factor. J. Exp. Med. 152:235.
6. Adorini, L., G. Doria, P. Ricciardi-Castagnoli. 1982. Fine antigenic specificity and genetic restriction of lysozyme-specific suppressor T cell factor produced by radiation leukemia virus-transformed suppressor T cells. Eur. J. Immunol. 12:719.
7. Okuda, K., M. Minami, D. H. Sherr, and M. E. Dorf. 1981. Hapten-specific T cell responses to 4-hydroxy-3-nitrophenyl acetyl. XI. Pseudogenetic restrictions of hybridoma suppressor factors. J. Exp. Med. 154:468.
8. Minami, M., K. Okuda, S. Furusawa, B. Benacerraf, and M. E. Dorf. 1981. Analysis of T cell hybridomas. I. Characterization of H-2- and Igh-restricted monoclonal suppressor factors. J. Exp. Med. 154:1390.
9. Kontianinen, S., E. Simpson, E. Bohrer, P. C. L. Beverly, L. A. Herzenberg, W. C. Fitzpatrick, P. Vogt, A. Torano, I. F. C. McKenzie, and M. Feldmann. 1978. T-cell lines producing antigen-specific suppressor factor. Nature (Lond.). 274:477.
10. Taussig, M., J. F. Corvalan, R. M. Binns, and A. Holliman 1979. Production of an H-2-related suppressor factor by a hybrid T-cell line. Nature (Lond.). 277:305.
11. Fresno, M., G. Nabel, L. McVay-Boudreau, H. Furthmayer, and H. Cantor. 1981. Antigen-specific T lymphocyte clones. Characterization of a T lymphocyte clone expressing antigen-specific suppressive activity. J. Exp. Med. 153:1246.
12. Okuda, K., M. Minami, S. Furusawa, and M. E. Dorf. 1981. Analysis of T cell hybridomas. II. Comparisons among three distinct types of monoclonal suppressor factors. J. Exp. Med. 154:1838.

13. Yamauchi, K., D. Murphy, H. Cantor, and R. K. Gershon. 1981. Analysis of an antigen specific H-2 restricted, cell free product(s) made by "I-J" Ly-2 cells (Ly-2 TsF) that suppresses Ly-2 cell depleted spleen cell activity. Eur. J. Immunol. 11:913.

14. Minami, M., K. Okuda, M. E. Sunday, and M. E. Dorf. 1982. H-2K-, H-2I-, and H-2D-restricted hybridoma contact sensitivity effector cells. Nature (Lond.). 297:231.

15. Baxevanis, C. N., Z. A. Nagy, and J. Klein. 1981. A novel type of T-T cell interaction removes the requirement for I-B region in the H-2 complex. Proc. Natl. Acad. Sci. USA. 78:3809.

16. Baxevanis, C. N., N. Ishii, Z. A. Nagy, and J. Klein. 1982. Role of the E^k molecule in the generation of suppressor T cells in response to LDH_B. Scand. J. Immunol. 16:25.

17. Baxevanis, C. N., N. Ishii, Z. A. Nagy, and J. Klein. 1982. H-2-controlled suppression of T cell response to lactate dehydrogenase B. Characterization of the lactate dehydrogenase B suppressor pathway. J. Exp. Med. 156:822.

18. Melchers, I., K. Rajewsky, and D. C. Shreffler. 1973. Ir-LDHB: map position and functional analysis. Eur. J. Immunol. 3:754.

19. Lemke, H., G. J. Hämmerling, and U. Hämmerling. 1979. Fine specificity analysis with monoclonal antibodies of antigens controlled by the major histocompatibility complex and by the Qa/TL region in mice. Immunol. Rev. 47:175.

20. Oi, V. T., P. P. Jones, J. W. Goding, L. A. Herzenberg, and L. A. Herzenberg. 1978. Properties of monoclonal antibodies to mouse Ig allotypes, H-2 and Ia antigens. Curr. Top. Microbiol. Immunol. 81:115.

21. Lerner, E. A., L. A. Matis, C. A. Janeway, Jr., P. P. Jones, R. H. Schwartz, and D. B. Murphy. 1980. Monoclonal antibody against an Ir gene product? J. Exp. Med. 152:1085.

22. Ozato, K., and D. H. Sachs. 1981. Monoclonal antibodies to mouse Mhc antigens. III. Hybridoma antibodies reacting to antigens of the H-2^d haplotype reveal genetic control of isotype expression. J. Immunol. 126:317.

23. Hiramatsu, K., A. Ochi, S. Miyatani, A. Segawa, and T. Tada. 1982. Monoclonal antibodies against unique I-region gene products expressed only on mature functional T cells. Nature (Lond.). 296:666.

24. Galfre, G., S. C. Howe, C. Milstein, G. W. Butcher, and J. C. Howard. 1977. Antibodies to major histocompatibility antigens produced by hybrid cell lines. Nature (Lond.). 266:550.

25. Corradin, G., H. M. Ettlinger, and J. Chiller. 1977. Lymphocyte specificity to protein antigens. I. Characterization of the antigen-induced in vitro T cell-dependent proliferative response with lymph node cells from primed mice. J. Immunol. 119:1048.

26. Elliott, B. E., Z. Nagy, M. Nabholz, and B. Permis. 1977. Antigen recognition by T cells activated in the mixed lymphocyte reaction: specific binding of the allogeneic cell material after removal of surface-bound antigen by trypsin. Eur. J. Immunol. 7:287.

27. Ishii, N., C. N. Baxevanis, Z. A. Nagy, and J. Klein. 1981. Responder T cells depleted of alloreactive cells react to antigen presented on allogeneic macrophages from nonresponder strains. J. Exp. Med. 154:978.

28. Nagy, Z. A., C. N. Baxevanis, and J. Klein. 1983. Cross-reactivity of suppressor T cells specific for lactate dehydrogenase B and IgG2a myeloma protein. J. Immunol. In press.

29. Elliott, B. E., B. Takacs, and Z. A. Nagy. 1979. Specific binding of radiolabeled membrane vesicles by T cells activated in the mixed lymphocyte reaction. Eur. J. Immunol. 9:646.

30. Germain, R. N., J. Theze, C. Waltenbaugh, M. E. Dorf, and B. Benacerraf. 1978. Antigen-specific T cell-mediated suppression. II. In vitro induction by I-J-coded L-glutamic acid^0L-tyrosine^0(GT)-specific T cell suppressor factor (GT-TsF) of suppressor T cells (Ts2) bearing distinct I-J determinants. J. Immunol. 121:602.
31. Yamauchi, K., D. Murphy, H. Cantor, and R. K. Gershon. 1981. Analysis of antigen specific Ig restricted cell free material made by I-J⁺ Ly-1 cells (Ly-1 Tsi) that induces Ly-2⁺ cells to express suppressive activity. *Eur. J. Immunol.* 11:905.

32. Tada, T., M. Taniguchi, and C. S. David. 1976. Properties of the antigen-specific suppressive T-cell factor in the regulation of antibody response of the mouse. IV. Special subregion assignment of the gene(s) that codes for the suppressive T-cell factor in the H-2 histocompatibility complex. *J. Exp. Med.* 144:713.

33. Yamauchi, K., N. Chao, D. B. Murphy, and R. K. Gershon. 1982. Molecular composition of an antigen-specific Ly-1 T suppressor inducer factor. One molecule binds antigen and is I-J⁺; another is I-J⁻, does not bind antigen, and imparts an IgG-variable region-linked restriction. *J. Exp. Med.* 155:655.

34. Flood, P., K. Yamauchi, and R. K. Gershon. 1982. Analysis of the interaction between two molecules that are required for the expression of Ly-2 suppressor cell activity. Three different types of focusing events may be needed to deliver the suppressive signal. *J. Exp. Med.* 156:361.

35. Steinmetz, M., K. Minard, S. Horvath, J. McNicholas, J. Frelinger, C. Wake, E. Long, B. Mach, and L. Hood. 1982. A molecular map of the immune response region from the major histocompatibility complex of the mouse. *Nature (Lond.)*, 300:35.