Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 3758–3762, May 1989
Immunology

Specific inhibition of cell-surface T-cell receptor expression by antisense oligodeoxynucleotides and its effect on the production of an antigen-specific regulatory T-cell factor

(suppressor T-cell factor/suppressor T cell)

HEGUANG ZHENG, BENI M. SAHAI, PATRICK KILGANNON, ARUN FOTEDAR, AND DOUGLAS R. GREEN

Department of Immunology, University of Alberta, Edmonton, AB, T6G 2H7, Canada

Communicated by Kimishige Ishizaka, January 6, 1989

ABSTRACT **We have used antisense oligodeoxynucleotides corresponding to genes encoding the variable (V) region of the T-cell receptor (TCR) α and β chains (V_α and V_β) to control TCR expression in T-cell hybridomas. Two hybridomas, A1.1 and B1.1, recognize a synthetic polypeptide antigen designated poly 18 {poly[Glu-Tyr-Lys-(Glu-Tyr-Ala)$_5$]} together with I-A^d. We have found that TCR function (production of lymphokines in response to antigen) and T3 expression were removed after protease treatment of the cells and were fully recovered 48 hr later. However, when antisense oligodeoxynucleotides corresponding to the appropriate TCR *V* genes were present after protease treatment, little or no recovery of TCR function or T3 expression was observed. This effect was specific for the TCR *V* genes utilized by the T cell: antisense oligodeoxynucleotides corresponding to the TCR *V* regions of A1.1 had no effect on TCR expression in B1.1 and vice versa. Thus, antisense oligodeoxynucleotides can be used to temporarily block expression of a TCR gene in a T-cell hybridoma. This technique was then applied to a paradoxical phenomenon in A1.1 cells. We had observed previously that A1.1 releases an antigen-specific immunoregulatory activity that shows the same antigenic fine specificity as is displayed by the TCR of A1.1. We now report that antisense oligodeoxynucleotides corresponding to the A1.1 V_α gene blocked the production of this soluble antigen-specific activity by the cell. Antisense oligodeoxynucleotides corresponding to A1.1 V_β, on the other hand, had no effect on the production of this antigen-specific activity. We discuss these observations in the context of recent findings on the nature of T cell-derived antigen-specific regulatory factors.**

T lymphocytes specifically recognize foreign antigen together with self major histocompatibility complex (MHC) molecules through the cell-surface T-cell receptor (TCR) complex. This complex is composed of the TCR α and β chains, which are responsible for antigen and MHC specificity (1), and the T3 molecules, which may be responsible for transducing the membrane signal (2). α Chain, β chain, and T3 are only expressed as a complex; in the absence of any one component, cell-surface expression does not occur (2).

A helper T-cell hybridoma (A1.1) has been described (3) that expresses TCR α and β molecules specific for a synthetic polypeptide designated poly 18 {poly[Glu-Tyr-Lys-(Glu-Tyr-Ala)$_5$]} and, in the presence of specific antigen and I-A^d, releases lymphokines. This T-cell hybridoma also constitutively produces a poly 18-specific cell-free factor involved in antigen-specific induction of suppression, which we call "suppressor-inducer factor" (4). Analysis has revealed (4) that the factor produced by A1.1 displayed the same antigenic fine specificity exhibited by the TCR on the A1.1 cell surface. Further, an anti-TCR antiserum was found to bind the antigen-specific factor (5). These results led us to speculate that at least some of the genes encoding the TCR α and β chains may be responsible for encoding the antigen-specific factor as well. To test this idea, we sought a method of specifically inhibiting the synthesis of the TCR α or β chains within the cell.

One approach to such specific inhibition is the use of antisense oligodeoxynucleotides. Antisense oligodeoxynucleotides corresponding to c-*myc*, when added to cultures of normal T lymphocytes, inhibit proliferation in response to mitogen (6, 7) and, when added to HL-60 cells, inhibit proliferation and induce differentiation (8). Antisense oligodeoxynucleotides corresponding to proliferating cell nuclear antigen similarly inhibit proliferation of BALB/c 3T3 cells (9). Therefore, we examined whether similar oligodeoxynucleotides could be used to inhibit synthesis of the TCR and the effect of such inhibition on production of the A1.1-derived antigen-specific regulatory activity.

MATERIALS AND METHODS

Animals. C57BL/10 mice were purchased from Ellerslie (Edmonton, Canada) and maintained in our facility at the University of Alberta.

Antigens. Sheep erythrocytes (SRBC) were purchased from Morse Biologicals (Edmonton). Poly-18, poly(Glu-Tyr-Ala), and K3K [Glu-Tyr-Lys-(Glu-Tyr-Ala)$_3$-Glu-Tyr-Lys] were synthesized and provided by B. Singh (University of Alberta).

Oligodeoxynucleotides. The α- and β-chain genes of the TCR expressed in A1.1 have been characterized by sequencing (P.K., Z. Novak, M. van Hoff, A. Fu, and A.F., unpublished data). A1.1 expressed the BW5147 TCR genes in addition to the α- and β-chain genes unique to A1.1. The A1.1 β-chain gene utilizes the $V_{\beta 6}$ segment (10) while the α-chain gene uses a member of the $V_{\alpha 1}$ family, identical to that of TA84 (11). The antisense oligonucleotides synthesized were complementary to the V_α and V_β leader sequence extending from the ATG codon to 22 or 23 nucleotides downstream. All oligodeoxynucleotides were synthesized by the Regional DNA Synthesis Facility at the University of Calgary and were not purified of contaminating $n-1$ and $n-2$ sequences, which were present at a concentration of $\approx$10%. The antisense oligodeoxynucleotides used are shown in Table 1.

T-Cell Hybridomas. The poly 18-specific T-cell hybridomas A1.1 and B1.1 have been described (3). The cells were maintained in RPMI 1640 medium supplemented with 10%

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.

Abbreviations: TCR, T-cell receptor; SRBC, sheep erythrocytes; pfc, plaque-forming cells; APC, antigen-presenting cells; Poly 18, poly[Glu-Tyr-Lys-(Glu-Tyr-Ala)$_5$]; K3K, Glu-Tyr-Lys-(Glu-Tyr-Ala)$_3$-Glu-Tyr-Lys; V, variable; FITC, fluorescein isothiocyanate; FACS, fluorescence-activated cell sorter; FCS, fetal calf serum.

Table 1. Antisense oligodeoxynucleotide sequences

Hybridoma	Sequence
A1.1	
$V_{\alpha 1.2}$ (antisense)	GTA AAA CAC TCA AGG ATT TCA T
$V_{\beta 6}$ (antisense)	CAG CAG AAA ACC CAC TTG TTC A (T)
B1.1	
$V_{\beta 2}$ (antisense)	GCA CAG AAT GCA AAA CTG CCA C (AT)

All antisense sequences used in the studies discussed herein correspond to the first 22 or 23 bases of the gene of interest, commencing with the translation start. Bases shown in parentheses were present in some but not all oligodeoxynucleotides used (no differences were observed between these variants). Concentrations of antisense oligodeoxynucleotides do not take $n - 1$ and $n - 2$ impurities into account (≈10%).

(vol/vol) fetal calf serum (RPMI 1640/10% FCS) and were recloned to ensure good responsiveness. Prior to exposure to oligodeoxynucleotides, the cells were treated with 0.25% trypsin (Sigma) for 10 min in a 37°C waterbath. Cells were then washed and cultured at 5×10^6 cells per ml in RPMI 1640/10% FCS with or without antisense oligodeoxynucleotides. Cells and supernatants were harvested after 48 hr.

Antigen Stimulation of T-cell Hybridomas and Lymphokine Assay. Poly 18-specific T-cell hybridomas were stimulated and tested for lymphokine production as described (3). T cells (5×10^4) were incubated with 1×10^4 irradiated antigen-presenting cells (APC; TA3 cells expressing I-$A^{d/k}$ exposed to 2000 R). Poly 18 or related peptide (3) (50 μg) was added as antigen. Cells and antigen were incubated in 200 μl of RPMI 1640/10% FCS in 96-well tissue culture plates (Costar) at 37°C in 94% air/6% CO_2. Supernatants were recovered after 48 hr and assessed for lymphokine by addition to CTLL-2 (2×10^4 cells per well). Proliferation of the lymphokine-responsive cells was assessed by [^{3}H]thymidine incorporation or 1-*N*-methyl-5-thiotetrazole staining.

Fluorescence Staining and Fluorescence-Activated Cell Sorter (FACS) Analysis. T cells were incubated with 145-2C11 [hamster anti-mouse T3 (12)] or with normal hamster serum on ice for 1 hr, washed, then incubated with fluorescein isothiocyanate (FITC)-labeled rabbit anti-hamster IgG (Nordic, Lausanne, Switzerland). H-2 K^k was assessed by using the 16.3.1N hybridoma (ATCC HB25) followed by FITC-labeled goat anti-mouse Ig (Kirkegaard and Perry Laboratories, Gaithersburg, MD). The cells were fixed with 1% formalin, and fluorescence was measured by FACS analysis.

Assay for Suppressor-Inducer Factor. Antigen-specific, suppressor-inducer activity from A1.1 was assessed as described (3). Supernatants from A1.1, cultured at 1×10^6 cells per ml (without antigen or APC), were collected after 48 hr and added at 10% to assay cultures. In addition, an "accessory supernatant" was prepared as follows: Splenic T cells from SRBC-immune C57BL/10 mice were prepared by using a T-cell recovery column (SciCan, Edmonton) and were cultured at 1×10^7 cells per ml for 48 hr; supernatants were collected from these cultures and adsorbed with 0.25 ml of packed SRBC per ml of supernatant on ice for 2 hr. The adsorbed "accessory supernatant" was added to assay cultures at 10%. Assay cultures contained C57BL/10 spleen cells (1×10^7 cells per ml) in RPMI 1640/10% FCS containing 50 μM 2-mercaptoethanol. In addition to accessory supernatant and/or A1.1 supernatant, cultures also contained 5×10^6 SRBC that had been conjugated with poly 18, poly(Glu-Tyr-Lys), or K3K by the method described by Mishell and Shigii (13). After 5 days of culture at 37°C in humidified 92% air/8% CO_2, anti-SRBC plaque-forming cells (pfc) per culture were determined (14).

RESULTS

Antisense Oligodeoxynucleotides Inhibit Reexpression of TCR Function in Trypsin-Treated T-cell Hybridomas. After trypsin treatment, A1.1 cells were found to optimally recover antigen and APC responsiveness after 48 hr in culture (data not shown). When trypsin-treated cells were cultured in the presence of 50 μM antisense oligodeoxynucleotides corresponding to the TCR *V* regions of A1.1, these cells failed to produce lymphokine upon exposure to specific antigen (Fig. 1). No effect was observed, however, when trypsin-treated A1.1 cells were cultured with a control V_β B1.1 antisense sequence. These effects were seen regardless of whether the lymphokines produced from the stimulated T cells were assayed by [^{3}H]thymidine incorporation or by 1-*N*-methyl-5-thiotetrazole staining of CTLL-2 cells (not shown).

The specificity of the observed effect of antisense oligodeoxynucleotide treatment was further tested by using a second poly 18-specific T-cell hybridoma, B1.1, which utilizes different TCR *V* region genes than does A1.1. Antisense oligodeoxynucleotides corresponding to TCR *V* regions were added to trypsin-treated A1.1 or B1.1 cells. After 48 hr, cells were recovered, washed, and exposed to antigen and APC. Lymphokine production was then assessed (Fig. 2). Inhibition of T-cell function was seen only when the appropriate antisense oligodeoxynucleotides were present in the cultures. Thus, the effect is *V* region-specific.

Since the expression of cell-surface T3 is dependent on the presence of TCR α and β chains (2), we also assessed the effects of the antisense oligodeoxynucleotides on reexpression of T3 after trypsin treatment. T3 was detected by staining with hamster anti-mouse T3 monoclonal antibody, followed by FITC-labeled rabbit anti-hamster Ig. Expression of T3 on A.1.1 or B1.1 cells was dramatically reduced by culture in the presence of the appropriate antisense oligodeoxynucleotides (Fig. 3). No cell-surface staining was detected with normal hamster serum followed by FITC-labeled rabbit anti-hamster Ig (not shown). In another experiment, we compared the reexpression of T3 versus H-2K^k on trypsin-treated A1.1 cells in the presence or absence of antisense oligodeoxynucleotides corresponding to $V_{\alpha 1.2}$. The presence of oligodeoxynucleotides did not affect reexpression of the H-2K molecule, while reexpression of T3 was completely inhibited (Table 2). Thus, the effects of the antisense oligodeoxynucleotide was specific for TCR.

Fig. 4 *Upper* shows that the optimal concentration of antisense oligonucleotide added to the trypsin-treated cells was 50 μM, although 25 μM produced significant effects on the reexpression of T-cell function. These results are entirely consistent with optimal concentrations of antisense oligonucleotides to c-*myc*, which inhibit T-cell mitogenesis (7). After 48 hr of exposure to 50 μM antisense oligodeoxynucleotides, some of the cells were washed and cultured for an additional 24 hr in the absence of the oligodeoxynucleotides. These cells

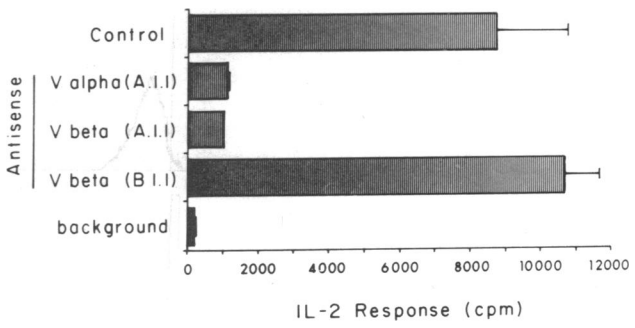

FIG. 1. A1.1 cells were typsin-treated and then cultured for 48 hr in the presence or absence of antisense oligodeoxynucleotide (50 μM) sequences, as indicated. Cells were harvested, washed, and placed into culture with antigen and APC; 24 hr later, supernatants were harvested and assayed for lymphokine [interleukin 2 (IL-2)] activity, expressed as incorporation of [^{3}H]thymidine by CTLL-2 cells.

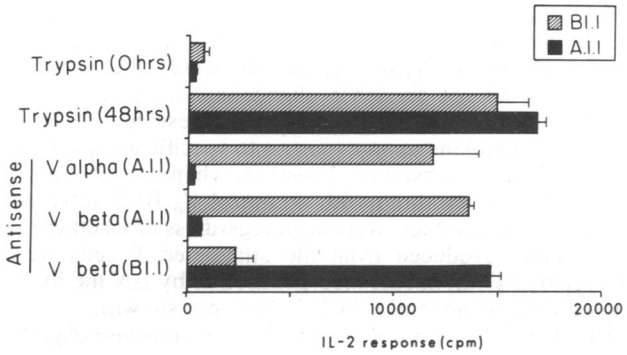

FIG. 2. A1.1 and B1.1 cells were trypsin-treated and cultured for 48 hr with the indicated antisense oligodeoxynucleotide (50 μM). Cells were harvested, washed, and tested for response to antigen and APC as in Fig. 1.

fully recovered their function (Fig. 4 *Lower*). This indicates that the specific oligodeoxynucleotides are nontoxic for the cells and that the effects are completely reversible.

Antisense Oligodeoxynucleotides Corresponding to V_α Inhibit the Production of an Antigen-Specific Regulatory Factor from A1.1. We had shown previously that supernatants of A1.1 contain a poly 18-specific regulatory activity that displays the same antigenic fine specificity displayed by the A1.1 cells themselves (4). To define the possible relationship between this antigen-specific regulatory activity from A1.1 and the TCR expressed by this cell, we cultured A1.1 cells in the presence of antisense oligodeoxynucleotides for 48 hr, collected supernatants, and assessed them for the presence of the regulatory activity. In the experiment shown in Fig. 5 *Upper*, A1.1 cells (no trypsin treatment) were cultured for 48 hr in the presence or absence of antisense oligodeoxynucleotides, and the supernatants were collected. The A1.1 supernatants with or without accessory supernatant were added to cultures of spleen cells and SRBC coupled with either K3K or poly(Glu-Tyr-Ala). K3K but not poly(Glu-Tyr-Ala) has been shown previously to interact with both A1.1 cells and the derived regulatory activity. Neither the A1.1 supernatants nor the accessory supernatant on their own had any effect on the subsequent anti-SRBC pfc response. When both the A1.1 supernatant and the accessory supernatant were added to cultures containing K3K–SRBC, a significant reduction in the subsequent anti-SRBC pfc response was observed. However, no effect was seen when poly(Glu-Tyr-Ala)–SRBC were present in the culture, demonstrating the antigen specificity of the effect. When the A1.1 cells were exposed to 50 μM antisense oligodeoxynucleotides corresponding to V_β of A1.1, there was no effect on the production

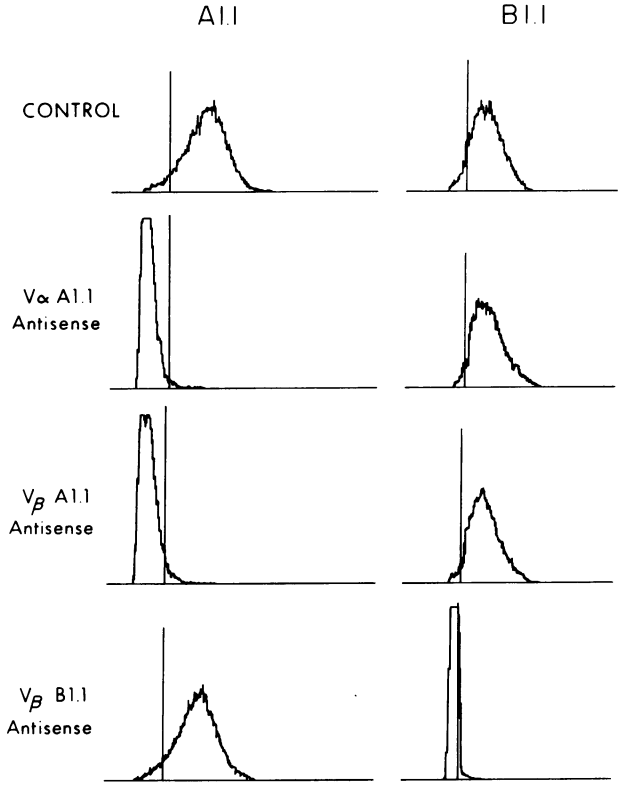

FIG. 3. A1.1 and B1.1 cells were treated as in Fig. 2. After 48 hr of culture, cells were harvested, washed, and stained with monoclonal hamster anti-mouse T3 (or normal hamster serum), followed by FITC-labeled rabbit anti-hamster Ig. Fluorescence staining was determined by FACS analysis.

Table 2. Antisense oligodeoxynucleotide corresponding to V_α inhibits reexpression of T3 but not H-2K

Antibody	Antisense in culture, % None	$V_{\alpha 1.2}$
Background	1.50	2.24
Anti-T3	99.50	1.95
Anti-H-2K^k	99.60	99.57

A1.1 cells were treated with trypsin and cultured for 48 hr in the presence or absence of 50 μM antisense oligonucleotide. Cells were recovered and stained with anti-T3 or anti-H-2K^k and a second FITC-conjugated antibody (see *Materials and Methods*). Background represents staining with the second antibody only. The percentage of positive cells were determined by FACS analysis.

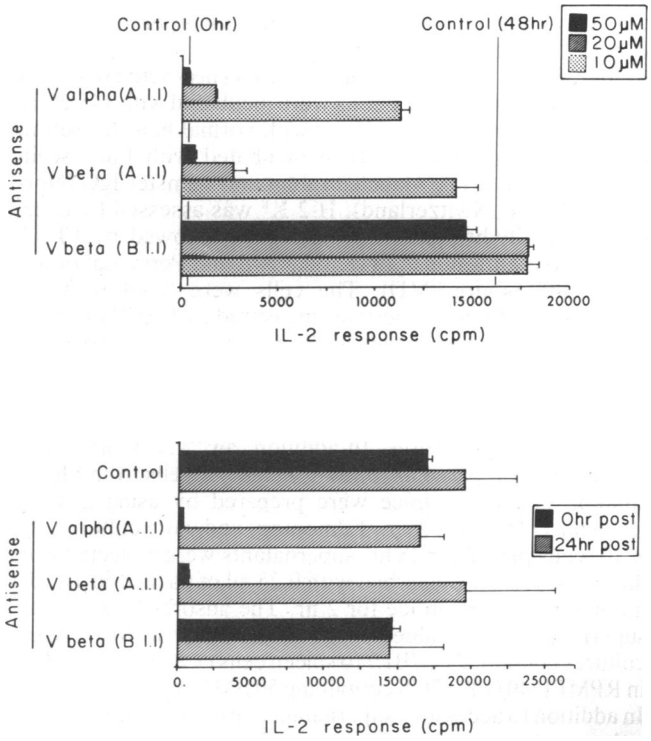

FIG. 4. (*Upper*) A1.1 cells were trypsin-treated and cultured for 48 hr in the presence of different concentrations of the indicated antisense oligodeoxynucleotides. Cell responsiveness was assessed as in Fig. 1. (*Lower*) Some of the A1.1 cells from the experiment in Fig. 5 *Upper* (treated with 50 μM of the indicated antisense oligodeoxynucleotide) were cultured an additional 24 hr in the absence of the oligodeoxynucleotides. Cells were recovered and assessed as in Fig. 1. Lymphokine production from the stimulated A1.1 cells, without the additional 24-hr culture (data from *Upper*), is shown for comparison.

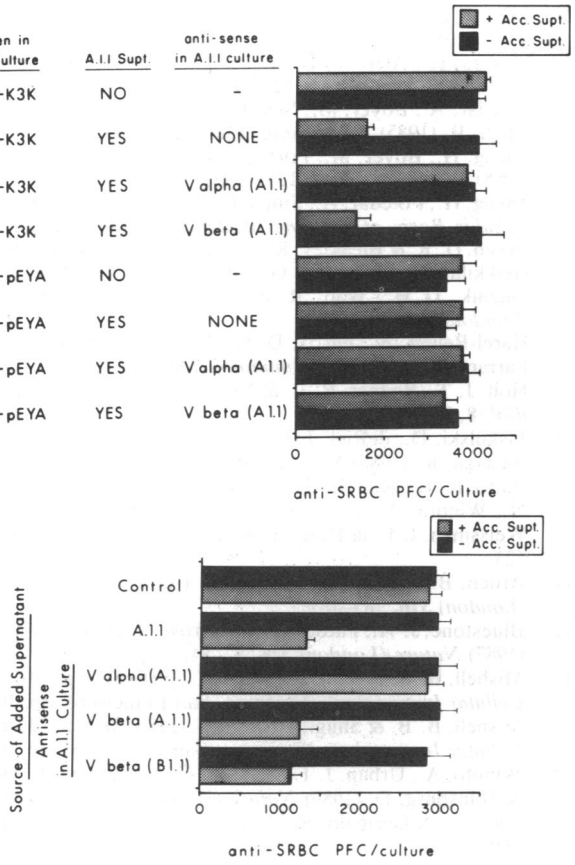

FIG. 5. Effect of antisense oligodeoxynucleotides on the constitutive production of an antigen-specific regulatory activity by A1.1. (*Upper*) A1.1 cells were cultured for 48 hr with or without the indicated antisense oligodeoxynucleotides (50 μM). The supernatants were harvested and tested for regulatory activity in the presence of SRBC coupled with either K3K or poly(Glu-Tyr-Ala). After 5 days, anti-SRBC pfc per culture were determined. (*Lower*) Trypsin-treated A1.1 cells were cultured with oligonucleotides, and the supernatants were assessed for regulatory activity as in *Upper* (antigen in the spleen cell assay culture was poly 18-conjugated SRBC).

of this antigen-specific activity. On the other hand, exposure of A1.1 cells to 50 μM antisense oligodeoxynucleotides corresponding to the V_α of A1.1 completely blocked the production of the factor. When supernatants of V_α antisense-treated A1.1 cells were added to spleen cell cultures containing accessory supernatants and K3K–SRBC, there was no reduction in subsequent anti-SRBC pfc.

Fig. 5 *Lower* shows a similar experiment, but in this case the A1.1 cells were treated with trypsin before exposure to antisense oligodeoxynucleotides, and the regulatory activity in the supernatants was assessed by poly 18–SRBC. Again, the constitutive production of the regulatory factor from A1.1 was inhibited by the presence of 50 μM antisense oligodeoxynucleotides corresponding to V_α of A1.1 but not V_β of A1.1 or V_β of B1.1. Antisense oligonucleotides to either V_α or V_β of A1.1 blocked reexpression of T3 as determined by FACS analysis, whereas no effect was seen of the corresponding V_β of B1.1 (results not shown but similar to those in Fig. 3). Thus, although the A1.1 cells treated with antisense oligodeoxynucleotides corresponding to the V_β of A1.1 failed to express cell-surface TCR, they nevertheless produced the regulatory factor.

In control experiments, no direct effects of the oligodeoxynucleotides on the anti-SRBC response or its suppression were observed (not shown). This is consistent with the lack of effect of the antisense oligodeoxynucleotides corresponding to V_β shown in Fig. 5. Therefore, the observed effects of the V_α antisense cannot be attributed to carry-over of the oligonucleotide into the assay culture. Thus, the constitutive production of the antigen-specific regulatory factor by A1.1 is blocked when the synthesis of TCR α chain is blocked but is unaffected by blocking TCR β-chain synthesis. These results are discussed below in the light of recent observations on the presence and possible role of TCR α chains in regulatory T-cell hybridomas.

DISCUSSION

Murine T-cell hybridomas, treated with trypsin, reexpress T3 and TCR function with time. When cultured in the presence of antisense oligodeoxynucleotides that correspond to the appropriate V_α or V_β sequences, this reexpression is inhibited (Figs. 1–4). The inhibition is *V*-region-specific (i.e., antisense must correspond to *V* regions used by the cell; Figs. 2 and 3) and is reversible (Fig. 4). Inhibition of reexpression of T3 had no detectable effect on the reexpression of an unrelated surface marker, H-2K (Table 2).

One interesting observation is the dramatic effect of antisense oligodeoxynucleotides on reexpression of T3 on the surface of appropriate cells (Fig. 3 and Table 2). This was somewhat surprising, as the TCR genes of the hybridoma fusion partner, BW5147, are expressed in the T-cell hybridomas used herein (P.K. and A.F., unpublished observation). Nevertheless, by blocking the expression of one chain of the TCR, T3 expression was virtually eliminated, even though the BW5147 TCR genes should have continued to be expressed. It is possible, for example, that in these hybridomas the different TCR chains (from BW5147 versus the original T cell) fail to associate in all combinations.

Herein, we have presented evidence that production of an antigen-specific, regulatory T-cell factor by A1.1 cells can be inhibited by specifically blocking TCR α-chain synthesis. The simplest explanation for this result is that the mRNA encoding the regulatory factor bears, at least, V_α. Alternatively, TCR α chain may be required for the release of the factor, without otherwise participating in the antigen-specificity of the activity. On the other hand, TCR β-chain synthesis does not appear to be required for the production of the factor. However, since $V_{\beta 6}$-specific antisense oligodeoxynucleotides appear to block $V_{\beta 6}$ but not $V_{\beta 2}$ expression (Figs. 2 and 3), we suspect that the BW5147 TCR β-chain (which bears $V_{\beta 1}$) continues to be expressed in the presence of antisense oligodeoxynucleotides corresponding to $V_{\beta 6}$. It is not excluded, therefore, that production of the A1.1-derived antigen-specific activity in the presence of $V_{\beta 6}$-specific antisense oligodeoxynucleotides depends on functional expression of the BW5147 TCRβ chain (as well as the A1.1 TCR α chain). If the antigen specificity of the A1.1-derived factor is indeed dictated by V_α, this suggests that in the A1.1 TCR, antigen-specificity is similarly dictated. This idea, that V_α controls antigen specificity of the TCR, has been suggested by some studies of TCR function (15, 16).

The regulatory activity from A1.1 participates in antigen-specific induction of suppression (4) and may be related to previously described T-cell suppressor-inducer factors (17–19). However, the factor from A1.1 is incomplete, requiring the "accessory supernatant" for function. Our analysis (4) suggests that the A1.1-derived activity probably represents the antigen-specific portion of such T-cell suppressor-inducer factors, while the accessory supernatant may provide the antigen nonspecific component (see, for example, ref. 19).

If antigen-specific regulatory T-cell factors are indeed dependent on the expression of V_α sequences, however, why do suppressor-inducer T-cell hybridomas appear to lack TCR gene rearrangements (20, 21)? A number of such hybridomas, analyzed for β-chain rearrangement, were found to have lost

the appropriate chromosome (chromosome 6) (21). On the other hand, TCR α-chain expression was found in a suppressor–inducer T-cell hybridoma (22), and both TCR α- and β-chain expression were found in a virally transformed suppressor inducer T cell (23).

A possible resolution of the controversy surrounding TCR in regulatory T cells may come from the recent results of Weiner and colleagues (24). They found that a very small population (1–3%) of $T3^+$ cells in their cloned suppressor-inducer T-cell hybridoma, F12, was responsible for all of the production of antigen-specific factor by this cell. Precipitation of T3 from this population revealed the presence of a heterodimer, likely to be the TCR. This indicates that the earlier results on the absence of TCR in regulatory T-cell hybridomas must be reevaluated. More recently, Kuchroo *et al.* (25) confirmed and extended these findings by using several Ts3 hybridomas. Selection for $CD3^+$ cells resulted in enhanced factor production and yielded populations capable of binding antigen. T3 on the surface of these cells was associated with a heterodimer that could also be precipitated with the antibody to the α-chain constant region (C_α). Modulation of T3 by anti-CD3 antibody reduced antigen binding to background levels, while modulation with the specific hapten significantly reduced T3 expression. It is possible, therefore, that the TCR α or β chains, or both, on the surface of these cells are capable of directly binding antigen.

Moorhead and colleagues (26) have similarly described a regulatory T-cell factor that appears to bear TCR determinants. This antigen-specific factor reacts with a rabbit anti-mouse TCR antiserum (26) that also reacts with the A1.1-derived factor discussed herein (5). In addition, a monoclonal anti-murine TCR α-chain antibody also appears to bind to the antigen-specific portion of the factor described by Moorhead (J. Moorhead, personal communication).

De Santis and colleagues (27) also have found a relationship between TCR genes and an antigen-specific regulatory T-cell factor. They found that mRNA from a virally transformed suppressor–inducer T cell could be hybrid-selected with TCR α- and β-chain genes, then translated *in vitro* to yield a two-chain antigen-specific factor (27). It would be interesting, in the light of our results, to determine whether the antigen-specific component of this factor is produced by the TCR α-chain gene-selected mRNA.

We have speculated previously that $CD4^+$ T cells, which bear a TCR with a sufficiently high affinity for antigen, may be directed into the suppressor pathway (by producing suppressor inducer molecules) upon exposure to antigen (5, 17, 28). Our results, reported herein, together with those of others discussed above, suggest that at least part of the resulting factor is intimately related to the TCR α molecule. It seems possible that we have taken a small step toward the long-awaited molecular characterization of antigen-specific T-cell regulatory factors.

We thank Ms. Rita Marcotte and Ms. Anna Fu for their expert technical assistance. This work was supported by grants to D.R.G. and A.F. from the Medical Research Council of Canada.

1. Saito, T., Weiss, A., Miller, J., Norcross, M. A. & Germain, R. N. (1987) *Nature (London)* **325,** 125–130.
2. Clevers, H., Alercon, B., Wileman, T. & Terhorst, C. (1988) *Annu. Rev. Immunol.* **6,** 629–662.
3. Fotedar, A., Boyer, M., Smart, W., Widtman, J., Fraga, E. & Singh, B. (1985) *J. Immunol.* **135,** 3028–3033.
4. Zheng, H., Boyer, M., Fotedar, A., Singh, B. & Green, D. R. (1988) *J. Immunol.* **140,** 1351–1358.
5. Zheng, H., Fotedar, A., Singh, B. & Green, D. R. (1989) in *The Cellular Basis of Immune Modulation*, eds. Kaplan, J. G., Green, D. R. & Bleakley, R. C. (Liss, New York), pp. 379–382.
6. Heikkila, R., Schwab, G., Wickstrom, E., Loke, S. L., Pluznik, D. H., Watt, R. & Neckers, L. M. (1987) *Nature (London)* **328,** 445–449.
7. Harel-Bellan, A., Ferris, D. K., Vinocour, M., Holt, J. T. & Farrar, W. L. (1988) *J. Immunol.* **140,** 2431–2435.
8. Holt, J. T., Redner, R. L. & Nienhuis, A. W. (1988) *Mol. Cell. Biol.* **8,** 963–973.
9. Jaskulski, D., deRiel, J. K., Mercer, W. E., Calabretta, B. & Baserga, R. (1988) *Science* **240,** 1544–1546.
10. Barth, R. K., Kim, B. S., Law, N. C., Hunkapiller, T., Sobiek, N., Winoto, A., Gershenfeld, H., Okada, C., Hansburg, D., Weissman, I. L. & Hood, L. (1985) *Nature (London)* **316,** 517–523.
11. Arden, B., Klotz, L. J., Siu, B. & Hood, L. E. (1985) *Nature (London)* **316,** 783–787.
12. Bluestone, J. A., Pardoll, D., Sharrow, S. O. & Fowlkes, B. J. (1987) *Nature (London)* **326,** 82–84.
13. Mishell, B. B. & Shiigi, S. M., eds. (1980) *Selected Methods in Cellular Immunology* (Freeman, San Francisco), pp. 108–109.
14. Mishell, B. B. & Shiigi, S. M., eds. (1980) *Selected Methods in Cellular Immunology* (Freeman, San Francisco), pp. 86–89.
15. Winoto, A., Urban, J. L., Lan, N. C., Goverman, J., Hood, L. & Hansburg, D. (1986) *Nature (London)* **324,** 679–682.
16. Saito, T. & Germain, R. N. (1987) *Nature (London)* **329,** 256–269.
17. Cone, R. E., Zheng, H., Chue, B., Beaman, K., Ferguson, T. & Green, D. R. (1988) *Int. Rev. Immunol.* **3,** 205–228.
18. Saito, T. & Taniguchi, M. (1984) *J. Mol. Cell. Immunol.* **1,** 137–145.
19. Flood, P. M., Lowy, A., Tominaga, A., Chue, B., Greene, M. I. & Gershon, R. K. (1983) *J. Exp. Med.* **158,** 1938–1947.
20. Hedrick, S. M., Germain, R. N., Bevan, M. J., Dorf, M., Engel, I., Fink, P., Gascoigne, N., Heber-Katz, E., Kapp, J., Kaufman, Y., Kaye, J., Melchers, F., Pierce, C., Schwartz, R. H., Sorensen, C., Taniguchi, M. & Davis, M. M. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 531–535.
21. Kronenberg, M., Governman, J., Haars, R., Malissen, M., Kraig, E., Phillips, L., Delovitch, T., Suciu-Foca, N. & Hood, L. (1985) *Nature (London)* **313,** 647–653.
22. Imai, K., Danno, M., Kimoto, H., Shigemoto, K., Yamamoto, S. & Taniguchi, M. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 8708–8712.
23. De Santis, R., Givol, D., Hsu, P., Adorini, L., Doria, G. & Apella, E. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 8638–8642.
24. Weiner, D. B., Liu, J., Hanna, N., Bluestone, J. A., Coligan, J. E., Williams, W. V. & Greene, M. I. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 6077–6081.
25. Kuchroo, V. K., Steele, J. K., Billings, P. R., Selvaraj, P. & Dorf, M. E. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 9209–9213.
26. Fairchild, R. L., Kubo, R. T. & Moorhead, J. W. (1988) *J. Immunol.* **141,** 3342–3348.
27. De Santis, R., Palmieri, G., Doria, G. & Adorini, L. (1987) *Eur. J. Immunol.* **17,** 575–578.
28. Green, D. R., Chue, B., Ferguson, T. A., Beaman, K. D. & Flood, P. M. (1987) *J. Mol. Cell. Immunol.* **3,** 95–108.