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Immunoregulation by mouse T cell clones III. Cloned H-Y-specific cytotoxic T cells secrete a soluble mediator(s) that inhibits cytotoxic responses by acting on both Lyt-2$^-$ and L3T4$^-$ lymphocytes

In this study we report that cloned Thy-1$^+$, L3T4$^-$, Lyt-1$^-$, Lyt-2$^+$, H-Y-specific and H-2D^b-restricted cytotoxic T cell lines (CTLL) when induced by lectin or antigen secrete a soluble mediator(s) (SF) that inhibits proliferation and generation of cytotoxic lymphocytes (CTL) in mixed lymphocyte cultures (MLC). The biological activity was separable by gel filtration and appeared as a broad peak in the molecular mass range between 10000 and 50000 kDa. It was found that the suppressive activity released by CTLL neither strictly correlates with their cytotoxic potential nor with their ability to produce immune interferon or lymphotoxin. SF was shown to elicit its activity in an antigen-nonspecific manner in that it suppressed the maturation of T lymphocytes responding to both, the appropriate H-Y antigen as well as to unrelated H-2^d alloantigens or to the hapten 2,4,6-trinitrophenyl (TNP). The effect of SF on CTL responses was most pronounced in early phases of primary or secondary MLC. When analyzed for its inhibitory activity on precursor cells in populations selected for either Lyt-2$^-$ or L3T4$^-$ lymphocytes, it was found that SF interfered with the maturation of both subsets. The inhibition of CTL responses elicited by SF could not be reversed by the addition of exogenous interleukin 2. The finding that SF also inhibited the proliferation of some but not all antigen-dependent cloned T cells with helper or cytotoxic potential provides evidence that the factor also may regulate effector lymphocytes. In addition, the results support the assumption that SF exerts its effect directly on the responder rather than the stimulator population, and demonstrate that the development of CTL from their precursor cells is controlled at least in part by the cytotoxic effector cells themselves via a soluble factor(s) that interferes with distinct stages of T cell maturation. These findings again emphasize the expression of multiple functions by CTL and indicate their possible role during the course of an immune response by their capability to eliminate target cells and to secrete a soluble product(s) that mediates feedback control.

1 Introduction

Regulation of both humoral and cellular immune responses by T cells involves complex interactions between subsets of lymphocytes with distinct functions. Recent investigations have demonstrated in antigen- and lectin-sensitized mouse lymphocyte cultures the existence of suppressor T lymphocytes (T_s, [1–13]) and of soluble suppressor T cell factors (SF, [14–17]) that inhibit proliferative responses in mixed lymphocyte cultures (MLC) and/or the generation of cell-mediated cytotoxicity. However, since in most studies heterogeneous populations of cells or the supernatants (SN) thereof were used it was difficult to associate the regulatory capacities observed with either an individual lymphocyte subset or with a distinct soluble mediator and to reveal the mechanism of their action. With the technique for establishing cloned T cell lines that is now available, a more effective analysis of the regulatory mechanisms operative in the development of cell-mediated immune responses can be accomplished. Recent studies have revealed that individual cloned T effector cells and their soluble products are able to elicit more than one biological activity which may even have opposite effects on the immune response [18–26].

We have previously demonstrated that lymphocytes from cloned T cell lines (CTLL) selected for cytotoxic function in addition expressed suppressor and/or helper activities for the generation of cytotoxic responses in an antigen-nonspecific manner [24]. We now report that sensitization of H-Y-specific CTLL by either lectin or antigen may lead to the production of a soluble mediator(s) that inhibits the development of cytotoxic lymphocytes (CTL) in culture. We show that this CTLL-derived soluble suppressive activity can be enriched by gel filtration and acts in an antigen-nonspecific way by interfering with the activation and/or expansion of precursor cells

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Abbreviations: **B6:** C57BL/6 mouse **B6pr:** Female C57BL/6 mouse, previously primed *in vivo* with male C57BL/6 spleen cells **MLC:** Mixed lymphocyte culture **MLR:** Mixed lymphocyte reaction **CTL:** Cytotoxic T lymphocyte **CTLP:** Precursor cell of CTL **CTLL:** Cloned CTL line **T_h:** T helper lymphocyte **T_hP:** Precursor cell of T_h **T_s:** T suppressor lymphocyte **SF:** Suppressor factor **SN:** Culture supernatant **CTLL-$SN_{Con\ A}$:** Culture supernatant from concanavalin A-activated CTLL **CTLL-SN_{Ag}:** Culture supernatant from antigen-activated CTLL **Con A-SN:** Supernatant from Con A-stimulated rat spleen cells **EL4-SN:** Supernatant from EL4 thymoma stimulated with phorbol myristate acetate **MLC-SN:** Supernatant from secondary mixed lymphocyte culture **IL2:** Interleukin 2 **TCGF:** T cell growth factor (lymphokine sources including IL2) **rec.hIL2:** Recombinant human interleukin 2 **IFN-γ:** Immune interferon **FMF:** Flow microfluorometry **PBS:** Phosphate-buffered saline **SC:** Spleen cells **PMA:** Phorbol myristate acetate **TNBS:** 2,4,6-Trinitrobenzene sulfonic acid **C:** Complement **[^{3}H]dThd:** Tritiated thymidine

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(T_hP, CTLP) of both T helper lymphocytes (T_h) and CTL as well as with the proliferation of some but not all cloned T cell lines. The data suggest that cytotoxic effector cells regulate the magnitude and duration of CTL responses by secreting lymphokine(s) that interfere with distinct steps of T cell activation.

2 Materials and methods

2.1 Mice, *in vivo* immunization

Adult mice of the strains C57BL/6 (B6), B10.D2, B10.BR, B10.A(4R), B10.A(5R), B10.MBR, DBA/2 were obtained from the animal colony maintained at the Max-Planck-Institut für Immunbiologie, Freiburg, FRG. Mice of both sexes were used between 6 weeks and 6 months of age. Female B6 mice were injected i.p. with 3×10^7 male B6 spleen cells (SC). Three weeks to 4 months later, SC from these mice were used for *in vitro* cultures.

2.2 Medium

RPMI 1640 medium (Gibco, Bonn, FRG) was supplemented with L-glutamine (2 mM), kanamycin (100 μg/ml), tylosine (10 μg/ml), HEPES buffer (25 mM), 2-mercaptoethanol (2×10^{-5} M) and 10% selected fetal calf serum.

2.3 Preparation of lymphokine sources

The preparation of supernatant (SN) from concanavalin A (Con A)-induced rat SC has been described in detail previously [27]. Briefly, Con A supernatant (Con A-SN) was prepared by stimulating SC from Spraque Dawley rats with Con A (5 μg/ml) at 5×10^6 cells/ml in RPMI 1640 culture medium. SN was collected after 24 h of incubation and supplemented with 20 mg/ml of methyl-α-D-mannoside (α-MM; Sigma Chemical Co., Munich, FRG). EL4 supernatant (EL4-SN) was obtained by stimulating a cloned subline of EL4 thymoma with 10 ng/ml phorbol myristate acetate (PMA) as described elsewhere [28]. Secondary MLC supernatant (MLC-SN) was prepared as described by Ryser et al. [29] by mixing 1×10^7 viable cells recovered from pools of 14-day-old primary BALB/c anti-DBA/2 MLC with 4×10^6 irradiated (2000 rds) DBA/2 SC in 10 ml of RPMI 1640 culture medium in 50 ml tissue culture flasks (Nunc, 16337, Wiesbaden, FRG). SN was collected after 24 h of incubation and sterilized by filtration.

2.4 Gel chromatography

For gel filtration of SF activity the SN was concentrated 10 times by filtration using an Amicon Ultra filter YM5 (cut off at 5000 Da; Amicon, Witten, FRG). Chromatography was performed at 4 °C using degassed phosphate-buffered saline (PBS) as running buffer. Sephadex G-200 (No. 17-0080-03, Pharmacia Fine Chemicals, Freiburg, FRG) was expanded and equilibrated in PBS. Two ml of concentrated SN were layered on top of the Sephadex G-200 columns (1.5 × 85 cm, Pharmacia) and eluted with PBS at a flow rate of 20 ml/h. The protein content of 60–70 3-ml fractions was determined using a spectrophotometer (Dual Path Monitor UV-2, Pharmacia) calibrated for absorbance at 280 nm. The column was calibrated with the molecular weight standards (Pharmacia): aldolase (158 000), bovine serum albumin (67 000), ovalbumin (43 000) and ribonuclease A (13 700). The void volume and column volume were determined with Blue Dextran 2000 and phenol red, respectively. For the test of SF activity, pools of three fractions (9 ml) were concentrated 18 times and added to the detection system at 10% and 2% final concentration, respectively.

2.5 Selection for lymphocyte subsets

For selection of Lyt-2$^-$ lymphocytes, SC were incubated with monoclonal anti-Lyt-2.2 antibodies (kindly provided by Dr. U. Hämmerling, Sloan Kettering Institute, New York, NY) and selected rabbit complement (C) as described in detail elsewhere [11]. Selection for L3T4$^-$ lymphocytes was achieved by treating SC with monoclonal anti-L3T4 antibodies (H-129-19.6, kindly provided by Dr. M. Pierres; Centre d'Immunologie, Luminy) and selected rabbit C. It was found that only few of the selected C sources tested were suitable for cytolysis in the presence of H-129-19.6 monoclonal antibodies.

2.6 Cloning of CTL

All cloned H-Y-specific cytolytic T cells were derived from long-term MLC containing female B6 responder T cells [prepared by nylon wool passage of SC derived from mice previously primed to male B6 cells (B6pr)] and male B6 stimulator cells as described [24]. Briefly, the H-Y-specific T cell line was restimulated *in vitro* in 50 ml tissue culture flasks (Nunc, 16337, Wiesbaden, FRG) at weekly intervals with 2.5×10^7 irradiated male B6 stimulator cells in RPMI 1640 medium in 5% CO_2 atmosphere. After 6 weeks the H-Y-specific T cell line was maintained and expanded in 50 ml tissue culture flasks together with antigen in Con A-SN-conditioned medium (10%). H-Y-specific CTL clones were obtained by limiting dilution procedures seeding 10 to 0.3 long-term culture responder cells/well on 5×10^5 irradiated (2200 rds) male B6 stimulator cells in Con A-SN-conditioned medium (10-20%) in round-bottom microtiter plates (Nunc, 163320). After 2-3 weeks, cytotoxic activities were tested in individual wells on ^{51}Cr-labeled Con A-stimulated male B6 target cells. Positive cultures from wells which had received either 1 or 0.3 cell/well were picked, expanded on antigen in the presence of Con A-SN in a volume of 2 ml in macrotiter plates (Linbro, New Hamden, CT, 1624 TC) and subsequently transferred to 50 ml tissue culture flasks for further growth. All CTLL were recloned twice on antigen and Con A-SN at dilutions of 0.3 cell/well under similar conditions. Two subclones, CTLL 1.2C5 and 1.3E6, were adjusted to grow in Con A-SN-conditioned medium in the absence of antigen and subsequently recloned by limiting dilution procedures as described above. Specificity and cytotoxic activity of CTLL were tested on Con A-stimulated SC of male and female B6 mice, on P815 tumor target cells and for lectin-mediated lympholysis on P815 tumor cells in the presence of PHA (Gibco, 670-0576; final concentration, 4% of stock solution).

2.7 Preparation of CTLL-derived factor

Lectin-induced factor(s) from CTLL (CTLL-$SN_{Con\ A}$) was prepared by sensitizing 1×10^6/ml H-Y-specific CTLL with 5 μg/

ml Con A for 3 h at 37 °C. Afterwards, the activated lymphocytes were washed three times in RPMI 1640 medium and subsequently cultivated for additional 24–48 h at 37 °C. SN was collected, supplemented with 20 mg/ml of α-MM and stored at –20 °C until use. For the generation of antigen-induced factor(s) from CTLL (CTLL-SN_{Ag}) 1×10^6/ml H-Y-specific CTLL were incubated with 5×10^6 irradiated male B6 SC previously treated with anti-Thy-1.2 plus C. After 24 h SN was collected and stored at -20 °C until use.

2.8 Assays for lymphokine activity in Con A-SN, EL4-SN, MLC-SN or in SN of cloned T cell

2.8.1 T cell growth factor (TCGF) activity

SN were tested for interleukin 2 (IL 2) activity by incubating serial dilutions of SN (50% initial concentration) with 2×10^3 cells of the lymphokine-dependent cytotoxic T cell line CTLL-2 (kindly provided by Dr. S. Gilles) in a total volume of 200 µl in round-bottom microtiter plates (Nunc, 163220). After 20 h of incubation, 1.25 µCi = 46.25 kBq tritiated thymidine ([^{3}H]dThd) was added for the last 4 h. Cells were harvested onto filter strips using an automatic cell harvester and counted in a liquid scintillation β counter. A standard titration of recombinant human IL 2 (rec.hIL 2, kindly provided by Dr. M. Wrann, Sandoz Forschungsinstitut, Wien, Austria) at dilutions ranging from 100 ng–60 pg was set up with each assay (1 arbitrary unit = dilution of IL 2 source (150–200 pg), at which CTLL-2 gave 50% of maximal incorporation of [^{3}H]dThd). The data were subjected to probit analysis, compared to the standard curve, and the activity of each sample was expressed in units of IL 2.

2.8.2 Immune interferon (IFN-γ) activity

SN were tested for IFN-γ activity by a plaque reduction assay in microtiter plates using vesicular stomatitis virus and L cells, as described [30]. Titers are expressed in laboratory units using as reference standard recombinant mouse IFN-γ (rec.IFN-γ; produced by Genentech, Inc., and kindly supplied by Boehringer, Ingelheim, FRG).

2.8.3 Lymphotoxin activity

SN were tested for cell growth inhibitory activity as described before [31] with slight modifications. Briefly, SN were added to L929 cells that had been seeded 24 h previously (8×10^3 cells/well) in 0.2 ml medium in flat-bottom microtiter plates (Nunc, 167008). [^{3}H]dThd was added for the last 5 h of a 72-h incubation period, samples were harvested onto filter strips using an automatic cell harvester and counted in a liquid scintillation counter. Inhibition was calculated as follows:

$$\% \text{ Inhibition} = 100 \times 1 - \frac{\text{cpm of L929 cultured with SN}}{\text{cpm of L929 cultured without SN}}$$

2.9 MLC and test of activities in CTLL-SN

MLC was set up by cultivating 1×10^6–3×10^6 responder SC together with 5×10^6 irradiated (2200 rds) stimulator cells (unmodified or modified with 2,4,6-trinitrobenzene sulfonic acid (TNBS, 3 mM, 10 min, 37 °C) in 2-ml in flat-bottom tissue culture plates (Linbro 1624 TL). Cells were cultured at 37 °C in a humified 5% CO_2 atmosphere. Various concentrations of CTLL-SN or SN from different sources were added to these cultures on day 0 to day 5 of the culture period. *In vitro* induced cell-mediated cytotoxicity (CML) was assayed on day 4 – day 6 on the appropriate ^{51}Cr target cells. For the simultaneous determination of proliferative responses, triplicates of 100 µl cell suspension were removed from the above cultures on day 3 to day 5 and pulsed for an additional 18 h by adding 1.25 µCi of [^{3}H]dThd/well. In experiments in which only proliferative responses were determined, cell cultures were set up in round-bottom microtiter plates (Nunc, 163320) containing 2×10^4–4×10^5 responder cells and 4×10^5 – 5×10^5 stimulator cells and various concentrations of SN from different sources in a volume of 0.2 ml. At day 3 or day 4 individual cultures were pulsed for the last 18 h of incubation with 1.25 µCi of [^{3}H]dThd per well. Cells were harvested onto filter strips using an automatic cell harvester and counted in a liquid scintillation β counter.

2.10 Assay for cell-mediated cytotoxicity

Specific ^{51}Cr release was determined by incubating serial dilutions of effector cells derived from macrocultures with a fixed number (2×10^3/well) of ^{51}Cr-labeled day 2 Con A-activated blasts from male or female B6 mice (unmodified or modified with TNBS), or with ^{51}Cr-labeled P815 tumor cells in a final volume of 0.2 ml for 4 h. Afterwards, plates were centrifuged and 100 µl of SN was removed for counting. The percentage specific lysis was calculated by using the equation: % ^{51}Cr release = $(x-y)/(z-y) \times 100$ in which (x) is cpm in the SN of target cells mixed with effector cells; (y) is cpm in the SN of target cells incubated alone; (z) is cpm after lysis of target cells in 1 N HCl.

3 Results

3.1 Characterization of T cell clones

The H-Y-specific CTLL used in this study were derived from long-term *in vitro* cultures by limiting dilution procedures as outlined in Sect. 2.6 and described in detail previously [24]. Five of them, 2.A4.1, 2.A4.2, 2.A4.3, 2.C10.1 and 2.E12.2, were maintained in the presence of antigen and Con A-SN and showed high to intermediate specific cytolytic activity on syngeneic male target cells (Fig. 1; CTLL 2.A4.2 with cytotoxic activity similar to that of 2.A4.1 and 2.A4.3 was not tested in this experiment). Two clones, 1.2C5 and 1.3E6, grew in the presence of Con A-SN only and lost their antigen-specific cytotoxicity (Fig. 1) but not their lytic potential [32]. When stained by indirect immunofluorescence with anti-Thy-1.2, anti-Lyt-1.2, anti-Lyt-2.2 and anti-L3T4 antibodies followed by FITC-conjugated second-step reagent and analyzed by flow microfluorometry (FMF), all 6 clones showed similar intensities for each of the three antibodies and expressed the phenotype Thy-1.2^+, $L3T4^-$, Lyt-1.2^-, Lyt-2.2^+ (data not shown). All antigen-dependent CTLL were restricted to H-$2D^b$ as shown in a representative experiment for the proliferative response of CTLL 2.A4.2 (Fig. 2). In contrast, CTLL 1.2C5 and 1.3E6 which were maintained in Con A-SN in the

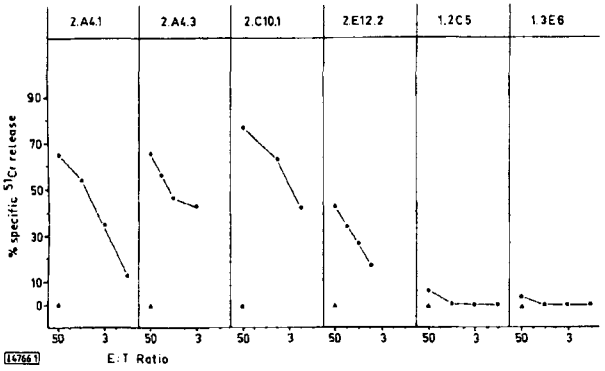

Figure 1. Cytolytic activity of H-Y-reactive CTLL. Individual cloned T effector cells were tested in a 4-h ^{51}Cr-release assay in various effector to target cell ratios on male B6 (●) or female B6 (▲) target cells. Numbers on the abscissa indicate effector to target (E:T) cell ratio. Percent specific lysis was calculated as described in Sect. 2.10.

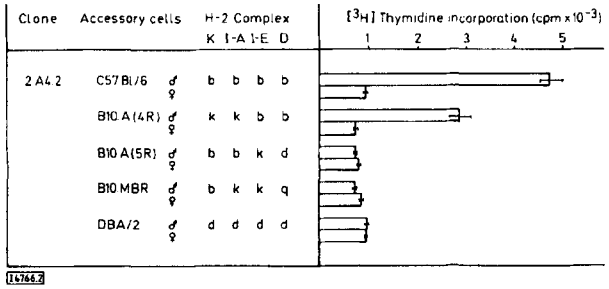

Figure 2. H-2 restriction specificity of H-Y-specific CTLL. Two × 10^4 cells from CTLL 2.A4.2 were sensitized with the indicated stimulator cells for 3 days. Proliferation was measured by [^{3}H]dThd incorporation.

absence of antigen showed comparable growth characteristics on all feeder cells, irrespective of their H-2 haplotype (data not shown).

3.2 Effect of CTLL-derived lymphokine(s) on the generation of CTL

SN derived from lectin-activated CTLL (CTLL-$SN_{Con\,A}$) were tested for their effect on the development of CTL *in vitro*. CTLL-$SN_{Con\,A}$ from different clones were added on day 0 to MLC of responder cells from female B6 mice previously primed to H-Y antigen (B6pr) and male B6 stimulator cells and the generation of CTL was assayed on day 6 of culture. Fig. 3 demonstrates that $SN_{Con\,A}$ from CTLL 2.A4.3 totally abrogated H-Y-specific cytotoxic responses at concentrations of 0.5 and 5%. In contrast, $SN_{Con\,A}$ derived from the other CTLL either reduced CTL responses only marginally (2.C10.1, 2.E12.2) or had no inhibitory activity at all (1.2C5, 1.3E6). Moreover, some of the CTLL-$SN_{Con\,A}$ (1.2C5, 1.3E6) even showed amplifying rather than suppressive activities when tested for their effect on the generation of H-Y-specific CTL under the same conditions (Fig. 3, panel 5, 6).

Fig. 4 illustrates that the amount of CTLL-$SN_{Con\,A}$ that was able to suppress the generation of H-Y-specific CTL varied considerably between the three subclones, 2.A4.3 (high),

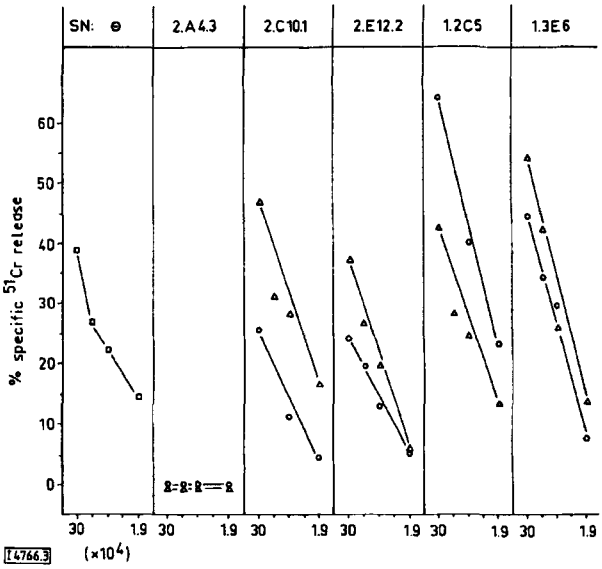

Figure 3. Effect of CTLL-$SN_{Con\,A}$ on the generation of H-Y-specific CTL. Three × 10^6 female B6pr responder cells were cultured with 5 × 10^6 male B6 stimulator cells in the absence (□, panel 1) or presence (panel 2–6) of 5% (○) or 0.5% (△) of $SN_{Con\,A}$ from the indicated H-Y-specific CTLL. After 6 days of incubation, effector activity was tested on 2 × 10^3 ^{51}Cr-labeled male B6 target cells. Numbers on the abscissa indicate the number of female B6pr responder cells (× 10^4) cultured on day 0, the descendants of which were tested on day 5. Percentage specific lysis was calculated as described in Sect. 2.10.

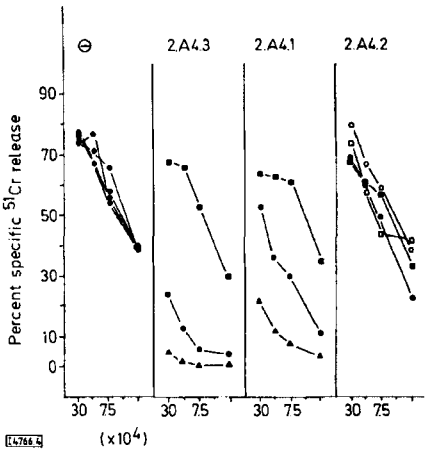

Figure 4. Effect of CTLL-$SN_{Con\,A}$ from three H-Y-specific T cell subclones on the generation of H-Y-specific CTL. Three × 10^6 female B6pr responder cells were cultured with 5 × 10^6 male B6 stimulator cells in the absence (●, panel 1; CTL activity in four individual culture wells) or presence (panels 2-4) of either 10% (▲), 5% (●), 1% (■), 0.5% (○) or 0.1% (□) of $SN_{Con\,A}$ from CTLL 2.A4.1, 2.A4.2 and 2.A4.3. After 6 days of incubation effector activity was tested on ^{51}Cr-labeled male B6 target cells. For numbers on the abscissa and percent specific lysis, see legend to Fig. 3.

2.A4.1 (intermediate) and 2.A4.2 (low), previously developed from the parental line CTLL 2.A4. Note that all clones showed similar cytotoxic activity when tested at the same time (Fig. 1). We have even observed that individual $SN_{Con\,A}$ preparations derived from the same CTLL 2.A4.3 contained variable amounts of SF activity (data not shown).

In Fig. 5, it is shown that SN from CTLL 2.A4.2 sensitized with male antigen (CTLL-SN_{Ag}) also contained SF that inhibited the development of H-Y-specific CTL (panel 1 *vs.*2). SN derived from a culture containing only male B6 stimulator cells did not show any suppressive activity (Fig. 5, panel 3). However, since the amount of SF activity found in different preparations of CTLL-SN_{Ag} varied considerably, only SN from lectin-activated CTLL were used in the following experiments.

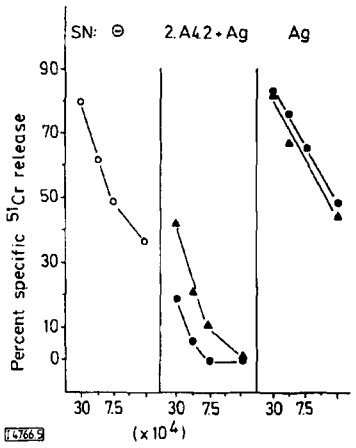

Figure 5. Effect of SN_{Ag} from CTLL 2.A4.2 on the generation of H-Y-specific CTL. Three × 10^6 female B6pr responder cells were cultured with 5 × 10^6 male B6 stimulator cells in the absence (○, panel 1) or in the presence of antigen SN_{Ag} (●, 20%; ▲, 5%) from CTLL 2.A4.2 (panel 2) or with SN (●, 20%; ▲, 5%) derived from cultures containing male B6 stimulator cells alone (panel 3). After 6 days of incubation effector activity was tested on ^{51}Cr-labeled male B6 target cells. For numbers on the abscissa and percent specific lysis, see legend to Fig. 3.

3.3 Antigen-specificity of SF activity and the susceptibility of developing CTL to the lymphokine(s)

Experiments were carried out to determine whether the effect of SF (CTLL-$SN_{Con\,A}$, 2.A4.3) is dependent on the presence of H-Y antigen(s) in MLC. As demonstrated in Figs. 6 and 7, the same CTLL-$SN_{Con\,A}$ suppressed similarly the generation of CTL responding to either the H-Y antigen (Figs. 6, 7), to TNP-modified syngeneic cells (Fig. 6, Ba) or to H-2^d alloantigens (DBA) (Fig. 7). These results clearly showed that the clonally derived factor acts in a nonspecific way.

To test the period of time in which MLC were susceptible to suppression, SF from CTLL 2.A4.3 was added at different times after culture initiation to responder cell cultures sensitized with either minor H-Y or major H-2 alloantigens (H-2^d). Fig. 7 shows that addition of SF (CTLL-$SN_{Con\,A}$, 2.A4.3) to MLC containing female B6pr responder cells and male stimulator cells on day 0 or on either day 1, 2 or 3 after initiation of culture resulted in total abrogation of cytotoxic

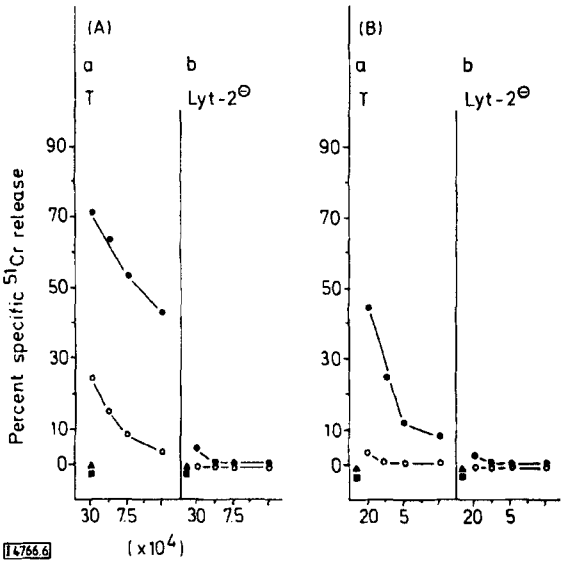

Figure 6. Effect of $SN_{Con\,A}$ from CTLL 2.A4.3 on the generation of H-Y (A) or TNP-(B)-specific CTL from unselected SC (a) and selected Lyt-2^- lymphocytes (b). Three × 10^6 unselected female B6 splenic responder cells (B6pr for anti-H-Y CTL responses, B6 unprimed for anti-TNP responses) or SC selected for Lyt-2^- lymphocytes by pretreatment with anti-Lyt-2 antiserum plus C were cultured with either male B6 or with TNP-modified syngeneic female B6 SC in the absence (●) or presence (○, 10%) of $SN_{Con\,A}$ from CTLL 2.A4.3. After 6 days of incubation effector activity was tested on either ^{51}Cr-labeled male B6 or syngeneic TNP-modified female B6 target cells. For numbers on the abscissa and percent specific lysis, see legend to Fig. 3. (▲) Cultures containing responder cells alone; (■) cultures containing stimulator cells alone.

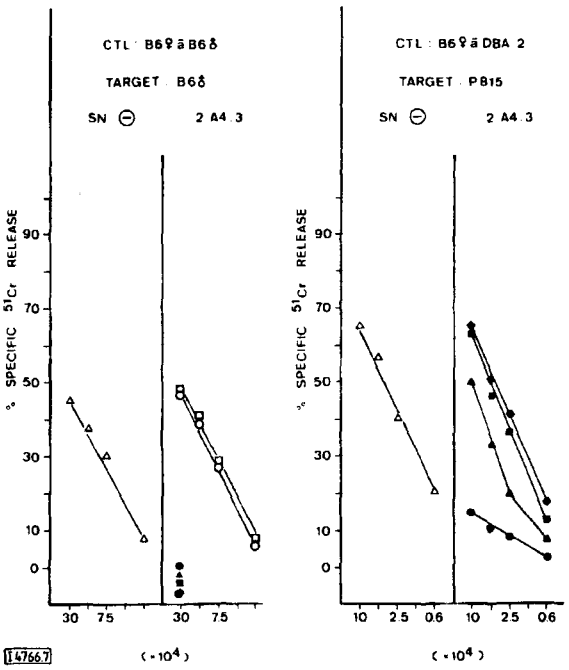

Figure 7. Susceptibility of anti-H-2^d and anti-H-Y CTL responses to suppression by $SN_{Con\,A}$ of CTLL 2.A4.3. Cultures contained either 3 × 10^6 female B6pr responder cells and 5 × 10^6 male B6 stimulator cells or 1 × 10^6 female B6 responder cells and 5 × 10^6 DBA/2 stimulator cells. $SN_{Con\,A}$ (10%) from CTLL 2.A4.3 was added once to these cultures on either day 0 (●) or day 1 to day 5, (▲, day 1; ■, day 2; ◆, day 3; ○, day 4; □, day 5) of the incubation period. Anti-H-2^d CTL response was tested on day 4 of culture on ^{51}Cr-labeled P815 tumor target cells, anti-H-Y CTL response was assayed on day 6 of culture on ^{51}Cr-labeled male B6 target cells. For numbers on the abscissa and percent specific lysis, see legend to Fig. 3.

responses (Fig. 7, panel 2). The addition of the SF source to similar cultures on day 4 or day 5 had no effect on the development of H-Y-specific CTL activity. In contrast, maximal suppression for the generation of alloreactive CTL by the same SF source was only seen when the lymphokine was added at day 0 of culture (Fig. 7, panel 4). Addition of SF to these cultures at day 1 or later had only marginal effects or did not interfere with the generation of CTL responses specific for H-2^d alloantigens.

3.4 Effect of exogenous TCGF on lymphokine-mediated suppression

The possibility that the effect exerted by SF on the development of CTL was due to the inhibition of TCGF production was tested in the experiment shown in Fig. 8. Female B6pr responder cells and male B6 stimulator cells were cultured with $SN_{Con\,A}$ derived from either CTLL 2.A4.3 or from CTLL 1.3E6 in the absence or presence of EL4-SN. Cultures were assayed on day 6 for CTL activity on male target cells. As shown before, $SN_{Con\,A}$ derived from CTLL 2.A4.3 but not $SN_{Con\,A}$ derived from CTLL 1.3E6 totally abolished CTL responses (Fig. 8, panel 3). Although the addition of EL4-SN to MLC considerably enhanced cytolytic activities (Fig. 8, panel 1 *vs.* 2) the same lymphokine source did not reverse the suppression of H-Y-specific CTL responses elicited by SF (CTLL 2.A4.3).

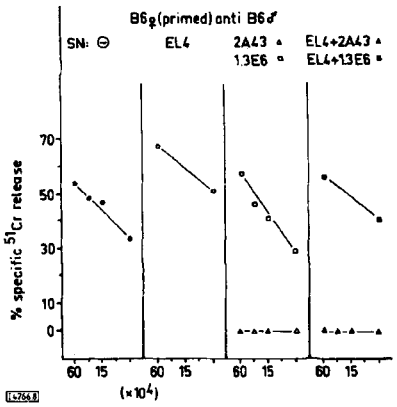

Figure 8. Effect of exogenous TCGF on suppression induced by CTLL-derived factor. Three × 10^6 female B6pr. responder cells were cultured with 5 × 10^6 male B6 stimulator cells alone (●, panel 1) or in the presence of either EL4-SN (10%, ○, panel 2), $SN_{Con\,A}$ from CTLL 2.A4.3 (△) or $SN_{Con\,A}$ from CTLL1.3E6 (10%, □, panel 3) or a mixture (5% + 5%) of EL4-SN with either $SN_{Con\,A}$ from CTLL 2.A4.3 (▲, panel 4) or $SN_{Con\,A}$ from CTLL 1.3E6 (■, panel 4). After 6 days of incubation effector activity was tested on ^{51}Cr-labeled male B6 target cells. For numbers on the abscissa and percent specific lysis, see legend to Fig. 3.

3.5 Effect of SF activity on the activation of Lyt-2^- and L3T4$^-$ T cells

We next examined the effect of CTLL-derived SF on the activation and/or expansion of selected T_hP and CTLP. Unprimed female B6 or B6pr responder cell populations were left untreated or were treated with anti-Lyt-2.2 antiserum and C and were incubated with either male B6 or TNP-modified female B6 stimulator cells for 6 days in the absence or presence of SF (CTLL-$SN_{Con\,A}$, 2.A4.3). As shown in Fig. 6, cytotoxic effector cells to either H-Y (A) or TNP (B) were only generated in cultures containing unselected lymphocytes but not in those which were negatively selected for Lyt-2^- cells. This observation is considered as a control for the proper elimination of CTLP. Proliferation of Lyt-2^- responder cells was assessed by incorporation of [^{3}H]dThd on day 3 or day 5 of culture. It is seen in Table 1 that in the presence of SF proliferative responses were considerably reduced in both unselected and selected Lyt-2^- responder populations cultured with male B6 stimulator cells. Furthermore, the inhibitory activity elicited by SF on the proliferative responses of both responder populations was antigen independent since suppression was also seen in lymphocyte populations responding to syngeneic and TNP-modified stimulator cells. Additional experiments showed that SF also interfered with the antigen-driven production of IL2 from Lyt-2^- lymphocytes and that the effect of SF on the proliferative responses of unselected and selected Lyt-2^- T cell populations was not reversed by exogenous sources of TCGF (data not shown).

To determine whether SF also interferes with the maturation of CTLP, we studied its effect on the population enriched for

Table 1. Effect of CTLL-$SN_{Con\,A}$ on the proliferation of unselected T cells and selected Lyt-2^- lymphocytes sensitized with either male or TNP-modified syngeneic stimulator cells[a)]

Responder	Treatment with antiserum to	Stimulator	SN 2.A4.3 (10%)	Incorporation of [^{3}H]dThd (cpm) day 3–4	day 5–6
B6pr	–	B6♂	–	14904 ± 730	32167 ± 2115
B6pr	–	B6♂	+	2589 ± 59	5307 ± 418
B6pr	Lyt-2.2 (+ C)	B6♂	–	6999 ± 488	6122 ± 55
B6pr	Lyt-2.2 (+ C)	B6♂	+	1515 ± 104	1889 ± 34
–	–	B6♂	–	235 ± 137	280 ± 24
B6♀	–	B6♀-TNP	–	19432 ± 1146	41585 ± 1409
B6♀	–	B6♀-TNP	+	4642 ± 441	9032 ± 396
B6♀	Lyt-2.2 (+ C)	B6♀-TNP	–	6866 ± 83	9529 ± 408
B6♀	Lyt-2.2 (+ C)	B6♀-TNP	+	1398 ± 206	1028 ± 49
–	–	B6♀-TNP	–	331 ± 173	32 ± 6

a) Unselected female B6 or B6pr responder cells (3 × 10^6) or the equivalent number of the selected Lyt-2^- lymphocyte populations were cultured with either 5 × 10^6 male B6 or with 5 × 10^6 TNP-modified female B6 stimulator cells. Aliquots of cultures were pulsed with [^{3}H]dThd on either day 3 or day 5 for the last 18 h of the incubation period as described in Sect. 2.9.

Table 2. Effect of $SN_{Con\,A}$ from H-Y-specific CTLL 2.A4.1 and 2.A4.3 on the induction of CTL from L3T4$^-$ lymphocytes[a)]

Responder population	Stimulator	Lymphokine source		Addition to culture (day)	Percent ^{51}Cr release from male target cells, Effector cells × 10^{-4} 30	15	7.5	1.9
Exp. 1								
L3T4$^-$	B6♂	–			5.6	0	0	0
		EL4-SN	(5%)	0	80.9	68.7	64.9	35.3
		EL4-SN + SF2.A4.1	(20%)	0	50.5	19.3	7.9	0
		EL4-SN + SF2.A4.1	(5%)	0	57.8	28.4	24.0	3.9
		EL4-SN + SF2.A4.3	(20%)	0	68.5	16.4	16.3	0
		EL4-SN + SF2.A4.3	(5%)	0	47.7	33.0	18.3	0
Exp. 2								
L3T4$^-$	B6♂	–			27.8	16.7	8.0	3.8
		EL4-SN	(5%)	0	78.4	72.6	59.5	41.0
		EL4-SN + SF2.A4.1	(20%)	0	26.8	19.5	13.8	3.0
		EL4-SN + SF2.A4.1	(20%)	1	80.2	70.5	59.2	39.8
		EL4-SN, SF2.A4.1	(20%)	0.1	72.8	64.0	60.0	35.9
		EL4-SN, SF2.A4.1	(20%)	0.3	77.2	67.2	62.8	40.1
		rec.hIL2 (100 U)		0	88.3	71.8	61.3	37.2
		rec.hIL2 + SF2.A4.1	(20%)	0	19.0	12.4	13.0	4.2
		rec.hIL2 (20 U)		0	51.7	35.6	29.9	10.0
		rec.hIL2 + SF2.A4.1	(20%)	0	17.4	11.7	6.2	2.3

a) Female B6pr responder cells (3×10^6) selected for L3T4$^-$ lymphocytes were cultured with 5×10^6 male B6 stimulator cells in the absence or presence of EL4-SN (5%) or rec.hIL2 (100 U/ml, 20 U/ml) and the indicated concentrations of CTLL-$SN_{Con\,A}$ (SF). Cytotoxic activity was tested on day 6 on ^{51}Cr-labeled male target cells. For the number of effector cells and percent specific lysis, see legend to Fig. 3.

this functional T cell subset. For this purpose responder cells from female B6pr mice were selected for L3T4$^-$ lymphocytes, known to be devoid of classical helper cells and to contain the majority of all CTLP ([33] and own unpublished results) by treatment with the appropriate monoclonal antibody (H-129-19.6) and C. The selected population was restimulated with male B6 SC in cultures supplemented with either EL4-SN or rec.hIL2 in the absence or presence of SF. The data in Table 2 (Exp. 1,2) illustrate that L3T4$^-$ lymphocytes did not develop into H-Y-specific CTL unless exogenous sources of TCGF (EL4-SN, rec.hIL2) were added. The admixture of SF from either CTLL 2.A4.1 or CTLL2.A4.3 to these cultures from the beginning resulted in significant reduction of cytolytic activities. On the other hand the addition of SF 1 or 3 days after the initiation of similar cultures or the admixture of both EL4-SN and SF 24 h after sensitization of L3T4$^-$ responder cells with antigen alone did not have any effect on the development of CTLL (Table 2, Exp. 2). Thus the data reveal that the susceptibility of L3T4$^-$ responder cells to suppression was confined to early stages of their maturation.

3.6 Effect of SF on the proliferation of cloned T cell lines with helper or cytotoxic potential

The previous experiments showed that in primary (anti-H-2^d) or secondary (anti-H-Y) *in vitro* immune responses SF exerted its effect mainly in the first days of culture suggesting a decrease of sensitivity of responder cells to suppression during their maturation. To test whether SF also interferes with the reactivation and/or expansion of effector cells, CTLL-SN was added to cultures of cloned T cell lines with helper or cytolytic potential. As shown in Table 3, two of the I-A^b-restricted T_h lines with specificity for the H-Y antigen, A3 and A5, were maintained on stimulator cells alone whereas another T_h line with similar antigen and H-2 restriction specificities (G8) and a H-Y-specific CTLL (2.A4.1) were cultivated on antigen in the presence of Con A-SN. It is seen that $SN_{Con\,A}$ from CTLL 2.A4.3 strongly inhibited the proliferation of T_h A5 (71%) but had only a marginal effect on the growth of T_h A3 (13%) or T_h G8 (11%). The same $SN_{Con\,A}$ also significantly suppressed the expansion of CTLL 2.A4.1 (36%). In the same experiment, $SN_{Con\,A}$ or SN_{Ag} from CTLL 2.A4.1 or 2.A4.2, respectively, showed only marginal or no suppressive activity at all. The observation that the proliferative responses of T_h A3 and T_h G8 was not inhibited by SF can be considered as an indication that the suppressive activity elicited by SF is not due to a mere toxic effect on the stimulator population.

3.7 Behavior of SF activity on gel filtration

Concentrated $SN_{Con\,A}$ from CTLL 2.A4.1 was subjected to gel chromatography. The eluted fractions were tested for suppressive activity on the proliferation of B6pr lymphocytes responding to male B6 stimulator cells. In Fig. 9 an activity profile of fractions eluted from Sephadex G-200 is depicted. SF activity was eluted as a broad peak ranging from about 10 to about 50 kDa. When $SN_{Con\,A}$ from CTLL 2.A4.3 was separated under similar conditions on a Sephadex G-75 column, SF activity eluted within the void volume (data not shown). It is possible that the wide molecular mass range of the clonally derived soluble mediator(s) as revealed by gel filtration is due to the presence of multiple factors.

3.8 SF activity does not correlate with the secretion of either IFN-γ or lymphotoxin

As shown in Tables 4 and 5, SN from lectin- or antigen-sensitized CTLL contained various amounts of the two lymphokines, IFN-γ (Table 4) and lymphotoxin (Table 5). In comparing the activity for the two factors with the suppressive

Table 3. Effect of SN from H-Y specific CTLL on the proliferative response of cloned H-Y specific T_h and CTL[a)]

Responding T cell (clone)	Culture conditions	Supernatant	Incorporation of [^{3}H]dThd (cpm)	Inhibition (%)
T_h A3	B6♂	–	7243	–
		2.A4.1 (Con A)	14828	0
		2.A4.3 (Con A)	6304	13
T_h A5	B6♂	–	7654	–
		2.A4.1 (Con A)	5976	22
		2.A4.3 (Con A)	2219	71
T_h G8	B6♂; Con A-SN	–	13183	–
		2.A4.1 (Con A)	12466	5
		2.A4.2 (Con A)	13935	0
		2.A4.2 (B6♂)	10787	18
		2.A4.3 (Con A)	11791	11
CTLL 2.A4.1	B6♂; Con A-SN	–	5958	–
		2.A4.1 (Con A)	6738	0
		2.A4.2 (Con A)	6738	6
		2.A4.2 (B6♂)	8349	0
		2.A4.3 (Con A)	3814	36

a) One × 10^4 of the indicated cloned T cells were incubated with 2×10^5 irradiated male B6 stimulator cells in the presence or absence of Con A-SN (10%). SN from mitogen (Con A) or antigen (B6♂) activated CTLL were added to the culture on day 0 at 10% final concentration. On day 2 [^{3}H]dThd was added for the last 6 h of incubation.

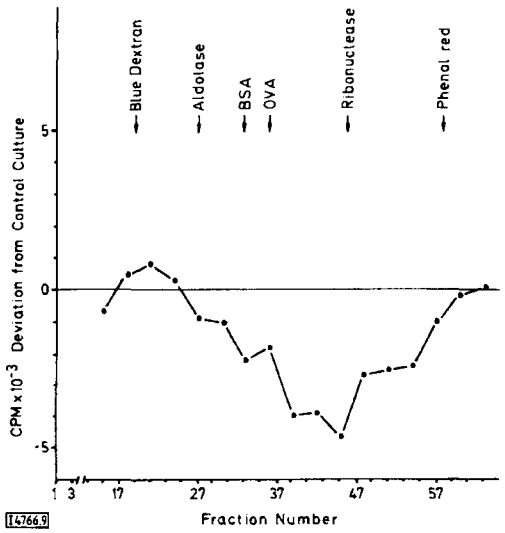

Figure 9. SF activity assayed after Sephadex G-200 gel exclusion chromatography. Tenfold concentrated CTLL-$SN_{Con\ A}$ from 2.A4.1 (2 ml) was layered on the column and the eluted fractions were tested for suppressive activity in MLC at 10% final concentration as described in Sects. 2.4 and 2.9. The effects on [^{3}H]dThd uptake in cultures containing B6pr responder cells and male B6 stimulator cells are shown. Data are plotted as deviations of cpm from cpm in control cultures set up in medium without CTLL-derived SN.

Table 4. IFN-γ activities in $SN_{Con\ A}$ or SN_{Ag} from H-Y-specific CTLL[a)]

Supernatant source	Stimulation with	Days of culture	IFN titer
1.2C5	Con A	1	81
1.3E6	Con A	1	9
2.C10.1	Con A	1	243
2.A4.1	Con A	1	81
2.A4.1	Con A	2	243
2.A4.2	Con A	2	243
2.A4.3	Con A	1	243
2.A4.3	Con A	2	243
2.A4.2	Ag	1	9
EL4-SN	–	–	3
Con A-SN	–	–	81
MLC-SN	–	–	27
rec.hIL 2	–	–	3

a) T cells from individual CTLL were cultured at 1×10^6/ml with either Con A or male B6 stimulator cells (2.5×10^6/ml) for the indicated time. SN was collected and tested for IFN-γ activity as described in Sect. 2.8.2.

activity described so far in individual SN, it became obvious that SF must be distinct from the other two lymphokines. This is indicated by the finding that $SN_{Con\ A}$ from CTLL 2.C10.1 and 2A4.1,2,3 contained similar titers of IFN-γ (Table 4) yet expressed quite different degrees of SF activity (see Figs. 3, 4). Moreover, lymphotoxin activity was highest in $SN_{Con\ A}$ of CTLL 2.A4.2 when compared with those in $SN_{Con\ A}$ from CTLL 2.A4.1 or 2.A4.3 (Table 5), yet SF activity was only detectable in the latter two (Fig. 4). In addition the experiments depicted in Tables 4 and 5 also reveal that there is no strict correlation between IFN-γ and lymphotoxin activities. The data therefore suggest that the three biological activities expressed by sensitized CTLL are the features of distinct soluble mediators.

Table 5. Effect of $SN_{Con\ A}$ from H-Y-specific CTLL on the proliferative response of L929 cells[a]

Source of supernatant from CTLL	[^{3}H]dThd incorporation in L929 cells cpm ± (SEM) 25% SN	12.5% SN	% Inhibition of L929 proliferation 25% SN	12.5% SN
Exp. 1				
–	195080 (9925)			
2.A4.1 (24 h)	103985 (2310)	97749 (4136)	47	50
2.A4.2 (48 h)	53367 (179)	67074 (803)	77	65
2.A4.3 (24 h)	106836 (836)	103215 (895)	45	47
1.2C5 (24 h)	131655 (2500)	117279 (1157)	32	40
Con A-SN	18095 (1528)	32035 (672)	91	84
EL4-SN	170712 (2980)	148346 (1700)	12	24
Exp. 2				
–	122360 (6470)			
2.A4.1 (24 h)	49422 (3201)	62519 (1610)	60	49
(48 h)	25154 (1549)	34012 (184)	79	72
2.A4.2 (48 h)	15156 (1798)	27484 (835)	88	77
2.A4.3 (24 h)	52741 (759)	63547 (1004)	57	48
(48 h)	42291 (2281)	44067 (2238)	65	64
EL4-SN	118085 (1649)	116316 (87)	3	5
Con A-SN	19124 (998)	42939 (350)	84	65
MLC-SN	35534 (880)	69457 (113)	71	43

a) T cells from individual CTLL were cultured at 1 × 10^6/ml with Con A for the indicated time. SN was collected and tested in the lymphotoxin assay as described in Sect. 2.8.3.

4 Discussion

It has been shown recently that cloned H-Y-specific CTLL may express multiple immunological functions: in addition to their cytolytic potential they are able to suppress or augment the development of cytolytic responses [24]. The experiments described here provide evidence that cloned CTL secrete SF activity that acts in an antigen-nonspecific fashion and inhibits the development of T cell cytotoxicity by preventing the activation and/or expansion of both T_hP and CTLP.

Production of SF could be induced in cultures of some but not all H-Y-specific Thy-1^+, L3T4^-, Lyt-1^-, Lyt-2^+ CTLL by sensitization with either Con A or the appropriate H-Y antigen (Figs. 3–5). The fact that SF was also found in SN of lectin-induced CTLL previously separated from residual stimulator cells argues against the remote possibility that cells other than CTLL are the source of this lymphokine(s). Secretion of SF by CTLL was more consistently found after lectin *vs.* antigen stimulation and in the latter case irradiation of CTLL prior to their activation increased the amount of SF detected in SN. These differences may be due to the requirement of particular membrane alterations for an efficient release of SF. However, other explanations are possible and further experiments are required to clarify this point.

The observation that different CTLL or even freshly established subclones with similar levels of cytotoxic activity produced quite different amounts of SF in response to Con A (Figs. 1, 3 and 4) is in line with our previous findings on the suppressive effects of lymphocytes from CTLL [24] and suggests that both activities are dissociated. It is possible that SF activity is only expressed at certain phases of the growth of CTL whereas the cytotoxic potential remains constant. Indeed, we were unable to find phase-dependent fluctuations in cytolytic activities of our CTLL (M. M. Simon, unpublished). This would also be an explanation for our findings that the amount of SF activity detected in different SN preparations from the same CTLL varied considerably. It is interesting that long-term culture T cell lines derived from H-Y-specific CTLL lost their specific cytotoxic activity (CTLL 1.2C5, 1.3E6) and the ability to produce SF under the conditions described herein (Fig. 3), though lymphocytes from CTLL 1.3E6 could still inhibit CTL responses ([24], and data not shown). Moreover, in some of the CTLL-$SN_{Con\ A}$ and CTLL-SN_{Ag} preparations, augmenting activities for the generation of CTL responses were found together with SF or in the absence of it (Fig. 3, and [24]). These results suggest that CTLL have the potential to secrete either simultaneously or alternating factors with multiple biological activities that can regulate CTL responses in a positive and/or negative way. The mechanism(s) leading to the secretion of the distinct soluble activities by an individual CTLL is unknown at present.

The suppression of MLC by SF was not antigen specific since it inhibited the proliferative and CTL responses to either the appropriate H-Y antigen or to unrelated antigens like TNP-modified syngeneic or H-2-incompatible cells. Nonspecific suppressive lymphokines with similar activities in CTL [15–17, 34] and/or proliferative responses [14, 15, 17, 34, 35] have been described before in SN of responder populations sensitized with either antigen [14-17] or Con A [34, 35]. However, since in all other studies total cell populations rather than cloned T cell lines were used, a direct comparison of these factors with our SF does not seem feasable. The SF activity described here was also not restricted to the H-2 haplotype of the responder population (data not shown) as reported for another SF (MLR SF) by Rich et al. [36]. However, we observed that the degree of SF-mediated inhibition of MLC varied for responder populations with different H-2 haplotypes (M. M. Simon, unpublished results).

A differential susceptibility of CTL responses to SF was seen in unselected lymphocyte populations responding to either

H-Y or to H-2^d antigens (Fig. 7). Whereas the H-Y-specific CTL responses with a maximum at day 6 of culture could still be inhibited by addition of SF to MLC on day 3, the generation of H-2^d-specific CTL with the maximum on day 4 of culture was only sensitive to SF when added at the beginning of culture but not later. It is possible that the differential effects of SF on both CTL responses is merely quantitative and a reflection of different numbers of precursor cells for T_h and CTL responding to either H-Y or H-2^d alloantigens ([37-39], and H. Moll, unpublished results). On the other hand the data could also be explained by assuming different kinetics for the activation of H-Y-specific compared to that of H-2-specific T_h and/or CTL with a concomitant prolongation of their sensitivity to suppression by SF. Although the observation that SF interferes with early events in the activation of H-Y-specific CTLP as well as preliminary experiments on the influence of SF on T_h maturation (data not shown) are in favor of the first assumption, a clear answer to this question cannot be given.

The experiments with lymphocytes selected for Lyt-2^- or L3T4$^-$ cells which are enriched for either ThP or CTLP, respectively, ([33], and own unpublished results) strongly suggest that SF interfered with the induction and/or differentiation of both T cell subsets. This was also supported by the findings that the production of IL 2 was inhibited together with proliferative responses in unselected or selected Lyt-2^- populations (Table 1, and unpublished results) and that exogenous sources of TCGF (EL4-SN, rec.hIL 2) did not reverse SF-mediated suppression of CTL responses (Table 2, Fig. 8). Although the exact sequence of events leading to the inhibition of CTLP maturation was not revealed in this study, it was shown that (a) TCGF-driven CTL responses in L3T4$^-$ lymphocytes were only suppressed when SF was present at the time of culture initiation, and (b) SF did not interfere with TCGF-dependent expansion of CTLP previously sensitized with antigen alone. These findings led to the conclusion that SF inhibits the development of both T_h and CTL from their precursors by interfering with antigen activation and/or early phases of CTLP differentiation rather than with their expansion.

Although the data discussed so far clearly showed that SF prevents the generation of effector cells from both T_h and CTLP, they did not address the question whether the targets of SF are the responder cells themselves or the stimulator population or both. Such an indirect suppressive effect via accessory cells cannot be formally excluded but seems unlikely, since the same SF preparation when tested on two individual antigen-dependent cloned T_h lines inhibited the proliferation of one but not the other. These observations are not only considered as a control to show that SF does not merely eliminate or inactivate the stimulator populations but they indicate in addition that effector cells can also be modulated by this lymphokine(s). Furthermore, the differential sensitivity of established T cell lines to SF suggests that the control mechanism exerted by this regulatory molecule on activated lymphocytes is restricted to certain growth phases.

The SF activity was separable biochemically and eluted from a Sephadex G-200 column as a broad peak with molecules ranging from 10 to 50 kDa thus excluding low molecular substances such as glucocorticoids, prostaglandins and cAMP which are known to interfere with T cell activation [40]. The data however do not reveal whether the wide range in the molecular mass of SF is due to the presence of different factors or to the association of related proteins. Interestingly, it has been shown recently that granules isolated from cloned T cell lines contain a set of unique proteins in the range between 14 and 75 kDa that may be responsible for cytolytic activity by a mechanism involving protein polymerization [41]. A similar phenomenon of assembling of proteins was also observed with a soluble mediator(s) with cytotoxic and suppressive activity derived from pig leukocytes ([42] and M. Kramer, personal communication). Thus it is possible that structures used by CTL for target cell lysis are also involved in suppression of immune responses as described herein.

We have shown that the cloned cytolytic T cell lines in addition to SF activity secrete to a various extent two other lymphokines, IFN-γ and lymphotoxin, which have been implied as regulatory molecules in the generation of CTL responses [31, 43, 44]. However, their biological activities did not coincide with the activity elicited by SF. It thus appears that the three biological activities secreted by individual CTLL are the characteristics of distinct factors. On the other hand, since the molecular mass of both lymphokines IFN-γ (17 kDa [45]) and lymphotoxin (25 kDa; D. V. Goeddel, personal communication) are related to the size of the lymphokine(s) with SF activity as separated from the column, it is possible that one or both of these factors contribute to suppression by acting in concert with other lymphokines.

In summary the observations in this study support the conclusion that factors from CTLL mediate suppression of T cell responses by interfering with the activation of precursor cells in both the T_h and CTL compartment. The inhibition of CTLP and T_h maturation seems to be confined to certain phases of maturation. Moreover, the data also reveal that CTLL-derived factors may also modulate the expansion of established T cell lines. Although the exact mechanism of SF remains to be determined the results suggest an effective control of the maintenance of cytotoxic responses by CTL. The data might have implications for the *in vivo* regulation of precursor and effector cells of CTL, *i.e.*, not only antigen elimination by CTL may negatively effect CTLP, but also the number of activated T cells may control via soluble mediators further generation and expansion of CTL.

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Rapid activation of ornithine decarboxylase by mitogenic (but not by nonmitogenic) ligands in human T lymphocytes*

The T cell mitogens, concanavalin A and the monoclonal antibody OKT3, cause a rapid activation of ornithine decarboxylase (ODC) activity in human T lymphocytes, maximal within 10 min of mitogen addition. Nonmitogenic ligands to T cell surface structures do not induce ODC. The enzyme induction is dependent upon an intact mobility of ligand-receptor complex, requires a functioning energy metabolism, but is independent of *de novo* protein synthesis. The early induction of pre-existing ODC molecules appears to be specifically linked to the initiation of T lymphocyte proliferation.

[I 4872]

1 Introduction

Various cells carry specific surface membrane receptors by which they can receive proliferation signals from the surrounding environment. Substances which induce DNA synthesis and cell division, such as mitogenic lectins, tissue-specific growth factors etc., have been identified. The cell stimulus system most extensively studied is the initiation of lymphocyte proliferation by mitogenic lectins. This phenomenon has been suggested to reflect the amplification event which is required to create efficient effector mechanisms during an immune response. The intracellular mechanisms which transfer signals from the triggered surface membrane receptors to the cell nucleus and thereby induce DNA synthesis are, however, still poorly understood.

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Abbreviations: **Con A:** Concanavalin A **HP-A:** Helix pomatia agglutinin **ODC:** Ornithine decarboxylase **[^{3}H]dThd:** Tritiated thymidine

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