Immunology 1988 **64** 439–443

Regulation of clonal growth by anti-T-cell receptor antibody-directed lysis

P. DE BERARDINIS, M. LONDEI, S. CARREL* & M. FELDMANN *Charing Cross Sunley Research Centre, Hammersmith, London, U.K. and *Ludwig Institute for Cancer Research, Epalinges, Switzerland*

Accepted for publication 2 March 1988

SUMMARY

CD4 and CD8 T-cell clones were generated using the Mx9 monoclonal antibody (mAb), which recognizes the $V\beta8$ T-cell receptor (TcR) gene family. The interaction of these clones with the Mx9 antibody was analysed and all were found to be specifically stimulated to proliferate by plastic-adherent Mx9. In the presence of Mx9 or its $F(ab')_2$ fragment, $CD8^+$ $Mx9^+$ clones were capable of specifically lysing the $CD4^+$ $Mx9^+$ T-cell clones. No lysis was seen of $Mx9^-$ T cells, or in the absence of the antibody. Conversely $CD4^+$ $Mx9^+$ T cells did not have lytic function. These results indicate that cross-linking of T cells via their antigen-specific receptors may initiate a unidirectional killing. Unlike previously reported lytic systems involving anti-TcR antibodies (e.g. anti-CD3), these results suggest that this mechanism may have an important physiological role in immune regulation. Anti-idiotypic antibodies have been shown to recognize T-cell receptors. These may exert profound immunosuppressive effects by inducing the lysis of the helper cells or B cells.

INTRODUCTION

There are many reports that anti-idiotypic antibodies may have profound effects on the immune system (reviewed by Burdette & Schwartz, 1987). The exact mechanism by which these antibodies exert these effects is not known. Idiotypic suppression may be very long lasting (e.g. monoclonal anti-T15 introduced neonatally in mice induces suppression that last for 26 weeks; Strayer *et al.*, 1974). However, it seems unlikely that the mere interaction of anti-idiotypic antibody with the B-cell surface would be sufficient to account for these profound effects.

A number of observations over the years have indicated that anti-idiotypic antibodies, raised against idiotypic determinants of Ig, may interact with T cells. For example Eichmann & Rajewsky (1975) were able to prime B and T cells with anti-nitrophenil NP anti-idiotype; Binz & Wigzell (1975) depleted alloractive T cells with anti-idiotypic antibodies to alloantibodies. These observations suggested that the profound effects of anti-idiotypic antibodies could be exerted by the intermediary action of T cells.

A number of groups have reported that cytotoxic T cells may be induced to lyse target cells that do not have the appropriate target antigen, provided their T-cell receptor becomes cross-linked to the target cell. Anti-T-cell receptor or anti-CD3 antibodies have been used to lyse Fc-receptor bearing target cells (Kranz, Tonegawa & Eisen, 1984; Mentzer *et al.*, 1985; Staerz & Bevan, 1985; Yssel *et al.*, 1986; Van Seventer *et al.*, 1987), and hybrid monoclonals used to cross-link T cells to tumour cells (Staerz & Bevan, 1985; Perez *et al.*, 1986; Jung, Ledbetter & Muller-Eberhard, 1987; Lanzavecchia & Scheidegger, 1987). These experiments suggest that anti-idiotypic antibodies could cross-link cytotoxic T cells to helper T cells or to B cells sharing the same idiotype, and induce their lysis. By this means anti-idiotypic (anti-receptor) antibodies could cause very dramatic and long-lasting immunoregulatory effects.

This hypothesis has been tested by using T-cell clones raised with an anti-receptor mAb, Mx9, which recognizes the T-cell receptor $V\beta8$ gene family. It was found that CD8 but not CD4 cells lysed T cells cross-linked to them by the Mx9 monoclonal or its $F(ab')_2$ fragment. This unidirectional lytic mechanism may be an important mechanism of immuneregulation mediated by anti-idiotypic antibodies *in vivo*, not by themselves but in concert with cytotoxic cells.

Abbreviations: CD, cluster differentiation; ^{51}Cr, chromium 51; CTL, cytotoxic T lymphocytes; EBV, Epstein–Barr virus-transformed B-cell line; FCS, fetal calf serum; IL-2, interleukin-2; mAb, monoclonal antibody; PBM, peripheral blood mononuclear cells; TcR, T-cell receptor; $V\beta$ variable portion of the β chain.

Correspondence: Dr P. De Berardinis, Charing Cross Sunley Research Centre, Lurgan Avenue, Hammersmith, London W6 8LW, U.K.

MATERIALS AND METHODS

Preparation of cells

Human peripheral blood mononuclear cells (PBM) were isolated from a healthy volunteer by Ficoll–Isopaque density gradient centrifugation (Pharmacia, Uppsala, Sweden).

Antibodies

Mx9 monoclonal antibody (mAb) is of the IgG1 subclass and recognizes Vβ8 gene products of the T-cell receptor, as previously described (Blanchard *et al.*, 1987; Carrel *et al.*, 1986). It was used at the optimal concentration (1:100 dilution from ascites). The other monoclonals used for this study were the following: Leu-4, (Becton-Dickinson, Mountain View, CA); OKT3, propagated from the American type culture collection (ATCC, Rockville, MD); Leu-3, anti-CD4 (Becton-Dickinson); Leu-2, anti-CD8 (Becton-Dickinson); 42/1C1, anti-Vβ5, kind gift of Dr A. Boylston; W6/32, anti-class I MNC, a kind gift of Drs J. and W. Bodmer; WT31, anti-αβ chain of TcR, kind gift of Dr W. Tax.

F(ab′)₂ fragments

These were prepared by pepsin digestion of the monoclonal antibody Mx9. Briefly, ascites was diluted 1:4 in 0·12 M acetate saline buffer, pH 3·8. Pepsin (EC 34 231; Sigma, Poole, Dorset) was added at the concentration of 1 mg/ml and the sample incubated overnight at 37°. The digested fractions were separated by HPLC (LKB TSKG3000SW column; Bromma, Sweden). Purity and size of the fractions were analysed by polyacrylamide gel electrophoresis and staining by Coomassie blue (LKB). No residual IgG was noted in the $F(ab')_2$ fractions. Subsequently the biological activity of the fragments was tested by immunofluorescence. Before use, the $F(ab')_2$ fragments were dialysed against phosphate-buffered saline (PBS; Sigma), pH 7.

Analysis and separation of lymphocyte populations with a fluorescence-activated cell sorter

For each analysis 2×10^5 T cells were incubated for 30 min at 4° with optimal concentrations of the following monoclonal antibodies: Leu-4, Leu-3, Leu-2 and Mx9. The cells were washed twice using PBS, then incubated with goat anti-mouse IgG FITC-conjugated (Southern Biotechnology, Birmingham, AL) at the working dilution of 1:100 for 30 min at 4°. After two more washes with PBS, 10,000 cells were analysed using a FACStar (Becton-Dickinson). In experiments to separate peripheral blood lymphocytes into $Mx9^+$ and $Mx9^-$ subpopulations, 2×10^7 cells were labelled with 1:100 dilutions of Mx9 ascites and then with fluorescein-conjugated goat anti-mouse Ig, and separated into $Mx9^+$ and $Mx9^-$ subpopulations using a FACStar.

EBV line

An autologous EBV line was produced by standard techniques using supernatant of the B95-8 EBV-producing marmoset line and cyclosporin A (Miller *et al.*, 1972). Briefly, 10^7 PBM were pelleted and resuspended in 1 ml of the B95-8 cell line supernatant filtered through a 0·2 μm filter. This preparation was incubated in a conical tube at 37°, resuspending occasionally. After 6 hr the cells were pelleted and resuspended at 10^6 cells/ml in RPMI-1640 plus 10% heat-inactivated FCS in the presence of 0·1 μg/ml of cyclosporin A (Sandoz, Basel, Switzerland). Half the medium was replaced every 3–4 days and outgrowth of EBV-transformed cells was evident after 4–6 weeks.

Establishment of T-cell clones

The cells separated by sorting were cultured in RPMI-1640 medium (Gibco, Grand Island, NY) supplemented with L-glutamine (2 mM), penicillin (100 U/ml), streptomycin (100 μg/ml), HEPES buffer (25 mM), 10% preselected human serum (blood group A or AB), and purified human recombinant IL-2 (Ajinomoto Japan, 20 ng/ml) at 37° in a humidified atmosphere of 5% CO_2 in air. After a week the cells were cloned by limiting dilution, 0·3 cell/well in Terasaki plates (Nunc, Kanmstrup, Denmark) (20 μl) in the presence of 10,000 autologous irradiated PBM (4000 rads, ^{137}CS source, Gammator B, Isomedix Inc., Parsippany, NJ), 20 ng/ml recombinant IL-2 and the optimal concentration of Mx9. At intervals of 1 week, the growing clones were transferred to 96-well round-bottomed plates, to 24-well plates and then into flasks, with 10^6 autologous PBM 1×10^6 (irradiated 4000 rads) as feeders, IL-2 and OKT3 at appropriate concentrations. The clones were then cultured in these conditions. Mx9-negative autologous clones were obtained by stimulation of PBM with an autologous irradiated (6000 rads) EBV-transformed B-cell line of the same individual for 1 week in the presence of IL-2 and then cloned by limiting dilution as above. The clones were grown in culture medium, in the presence of autologous irradiated PBM, recombinant IL-2 and OKT3 (Londei *et al.*, 1988).

Proliferative assay

Saturating concentration of the monoclonal anti-TcR or CD3 antibodies Mx9, WT31, UCHT1, 42/1C1 or OKT3 were coated to the plastic surface of each well of a 96-well round-bottomed microculture plate by incubation for 1 hr at 37°. Excess antibody was eliminated by two washes with PBS and 25,000 T cells, 7 days after addition of the feeder mixture, were added in 200 μl culture medium. After 3 days of incubation, 1 μCi tritiated thymidine (Amersham, Bucks, U.K.) was added to each well. Eight hours later the cells were harvested using a semi-automatic cell harvester onto glass fiber strips and the amount of incorporated [^{3}H]thymidine was assessed by liquid scintillation counting. The results are presented as the arithmetic mean of triplicate cultures.

Cytotoxicity assays

Cytotoxic activity was determined using a ^{51}Cr-release assay. Ten thousand ^{51}Cr-labelled target cells in 200 μl RPMI-1640 with 10% AB^+ human serum were mixed with effector cells in U-shaped wells of a 96-well microtitre plate, and incubated for 6 hr at 37° in a humidified atmosphere of 5% CO_2. The supernatants were harvested and counted in an LKB gamma counter. Each effector to target cell interaction was measured in triplicate and the data are presented as percentage specific ^{51}Cr release:

$$^{51}\text{Cr-release} = \frac{\text{experimental release} - \text{SR}}{\text{MR} - \text{SR}} \times 100.$$

The maximum release (MR) was determined after incubation of the cells in 5% NP40 and the spontaneous release (SR) by measuring the release by target cells in medium only. To determine the capacity of mAb to affect the cytotoxic reaction, effector or target cells were pre-incubated with mAb for 30 min at 4° and washed twice. The cells were then mixed and the ^{51}Cr-release assay was carried out as described above.

RESULTS

Fluorescence analysis and separation of lymphocytes

Figure 1 shows the results of the separation of PBM by cell sorting (see the Materials and Methods). Post-sort viability was

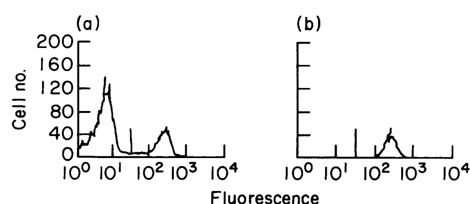

Figure 1. Immunofluorescence analysis of PBM before (a) and after (b) separation using a FACStar cell sorter. Cells were stained with Mx9 mAb followed by goat anti-mouse Ig FITC. A $Mx9^+$ population was identified (a, right). After separation by sorting a purified population more than 95% Mx9-positive was obtained (b)

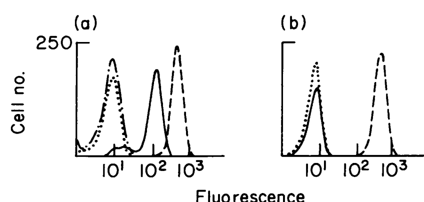

Figure 2. Immunofluorescence staining of positive (a) and negative (b) sorted cells after 1 week in culture. T cells were stained with anti-CD3 (--), Mx9 (-), 42/1C1 (. .), and a mAb of irrelevant specificity of the same class and species of the others (- . -) used as a control.

greater than 95% as judged by trypan blue exclusion. Purity of the separated T-cell subsets was in excess of 95% (Fig. 1b). Positive and negative separated populations were kept in culture and after 7 days they were phenotyped again. The positive sorted cells were >95% $CD3^+$ and $Mx9^+$, but all negative when stained with an anti-$V\beta5$ monoclonal antibody or an irrelevant control mouse antibody of the same Ig class (IgG1) as the others (Fig. 2a). Conversely the negatively sorted cells were $CD3^+$ but $Mx9^-$ (Fig. 2b).

Table 1. Proliferative response with antibody bound to plastic

Clone*	Mx9	WT31	42/1C1	OKT3
1CD4	78617	52058	499	79158
2CD4	84583	47055	578	73337
3CD8	83198	50573	602	65740
4CD4	91115	49896	657	60661
5CD4	87716	54616	589	71842
6CD8	84003	41411	606	82429
7CD8	82602	54883	623	62364
8CD8	92719	45861	609	70354
9CD4	89324	50413	703	91529
10CD4	81073	43103	729	78435
11CD4	89854	57700	662	60816
12CD4	94058	44273	488	73850
13CD4	97290	44402	544	72990
14CD4	71517	54894	546	63715
15CD4	93412	42496	964	81371
16CD4	81127	53681	474	67402
17CD4	85612	42328	430	61972
18CD4	79581	59492	643	63211
19CD4	93817	45384	657	79831
20CD8	88455	46643	687	61417
21CD4	87912	50719	770	77335
22CD4	82454	57873	538	66244
23CD4	80004	50611	655	75926
24CD4	78549	51738	527	61325
Means ± SD of 24 clones =	85774±6255	49712±5297	615±112	70968±8525

The antibodies were coated to the surface of a 96-well round-bottom microculture plate by incubation for 1 hr at 37°. Excess antibody was eliminated by two washes with PBS.

Twenty-five thousand T cells were added in 200 μl culture medium. Proliferation was measured by tritiated thymidine incorporation (c.p.m.) after 3 days of culture.

The data are expressed as the mean of triplicate culture.

* Clone designation indicates surface phenotype.

Clones

After 1 week of culture in IL-2-containing medium, the positive-sorted cells were cloned by limiting dilution in the presence of Mx9 monoclonal antibody. One-hundred and nine clones were maintained and cryopreserved, of which 24 were analysed. Phenotypic characterization of the clones showed that all of them expressed Mx9 and CD3, 19 were $CD4^+$ and five $CD8^+$.

Proliferative response with antibody bound to plastic

All the CD4 and CD8 clones showed a strong proliferative response with the antibodies Mx9, WT31 and CD3. In contrast neither CD8 or CD4 clones responded to the antibody 42/1C1, which reacts against the $V\beta5$ gene products of the TcR (Table 1).

Cytolytic activity of the clones

On co-culturing the $Mx9^+$ CD8 clones did not kill either the $Mx9^+$-related CD4 clones or $Mx9^-$ autologous clones (Fig. 3). To analyse the effect of anti-TcR or anti-CD3 mAb on the cytotoxic reaction, the targets cells were first incubated with the mAbs (see the Materials and Methods) and then with the

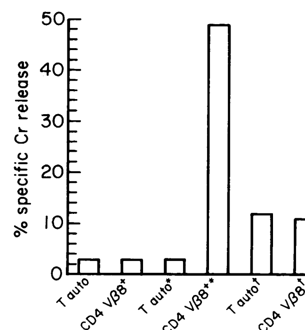

Figure 3. Effects of Mx9 (*) and OKT3 (†) mAbs on the lysis of CD4 clones by CD8 clones. CD4 target cells were labelled with ^{51}Cr and incubated with Mx9 (1:100 dilution from ascites or OKT3 (1 μg/ml) for 1 hr at 4° and washed twice. The E:T ratio was 1:10. The other ratios tested but not shown in the figure were 1:3 and 1:30, and at all ratios the same pattern was observed. High levels of cytolysis were seen only against CD4 $Mx9^+$ target cells in the presence of Mx9 mAb(*). A smaller cytotoxic reaction was observed either with OKT3 bound to CD4 $Mx9^+$ clones (†) or bound to autologous CD4 $Mx9^-$ clones (†).

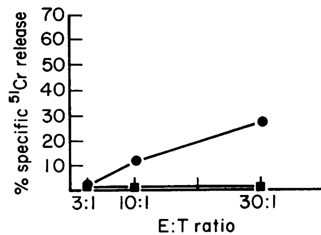

Figure 4. Effect of mAb Mx9 bound to effector cells CD8 Mx9$^+$ on the cytotoxic activity towards CD4 Mx9$^+$ targets (●) and autoreactive CD4 Mx9$^-$ targets (■).

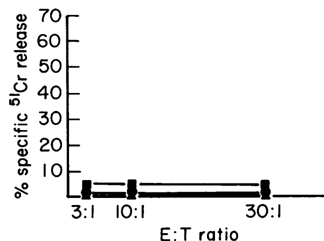

Figure 5. Lack of cytotoxic activity of CD4 effector against CD8 (■) or CD4 (●) targets coated with Mx9 mAb. Specific release was less than 3%.

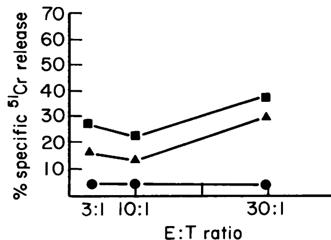

Figure 6. Effect of Mx9 mAb (■), F(ab′)$_2$ fragment of Mx9 (▲), and W6/32 mAb (●) bound to CD4 Mx9$^+$ target cells. CD8 Mx9$^+$ CTL clones were used as effector cells. Specific release at E:T ratio 30:1 was more than 20% when the Mx9 or the F(ab′)$_2$ fragment were used. F(ab′)$_2$ fragments were obtained after pepsin digestion and purification with HPLC, and the size of the eluted fraction was checked by polyacrylamide gel electrophoresis. When the anti-class I mAb W6/32 was bound to target cells specific release was less than 30%.

effector cells at different effector: target (E:T) ratio. As shown in Fig. 3, in the presence of the Mx9 mAb only CD4 Mx9$^+$ target cells were killed by CD8 clones. In contrast, using OKT3, both Mx9$^+$ and autologous Mx9$^-$ CD4 clones were killed, but to a lesser extent than with Mx9 mAb. The binding of Mx9 mAb to the effector cells (CD8 Mx9$^+$) also resulted in the killing of CD4 M x 9$^+$ cells only (Fig. 4). Furthermore, a cytotoxicity assay was performed using CD8 or CD4 clones as target cells and CD4 clones as effector cells. No cytotoxic action was demonstrated even in a 18-hr assay. In this experiment all the CD4 clones ($n = 19$) were examined (one example is shown in Fig. 5).

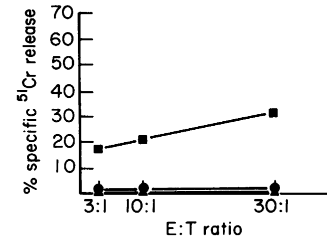

Figure 7. Effect of mAb Mx9 (■) or F(ab′)$_2$ fragment of Mx9 (●) bound to the EBV-transformed B-cell line of the same individual. CD8 Mx9$^+$ CTL were used as effector cells.

Conversely CD8 effectors also killed CD8 target cells, but the percentage of chromium released by CD8 target cells was less than the amount usually released by CD4 target cells (15% of specific chromium release of CD8 Mx9$^+$ cells, c.f. 56% CD4$^+$ Mx9$^+$ in a typical experiment at the E:T ratio of 30:1).

Type of antibody needed to induce cytolysis

In order to determine whether the killing effect of CD8$^+$ clones involves binding to the Fc receptor, or to the TcR, the assay was performed in the presence of the F(ab′)$_2$ fragment of the Mx9 mAb (Fig. 6). In this experiment CD8$^+$ clones lysed CD4 M x 9$^+$ clones bound with the F(ab′)$_2$ fragment. In contrast the binding of the F(ab′)$_2$ fragment to CD8 effector cells did not induce the lysis of the EBV-transformed B-cell line of the same individual (Fig. 7). This verifies that there was insignificant amounts of undigested Mx9 IgG left in the F(ab′)$_2$ preparation. The action of an anti-class I antibody (W6/32) was analysed. No killing was seen by CD8 clones in the presence of the anti-class I antibody (Fig. 6), indicating that cross-linking of cells clones was not sufficient and that T-cell receptor activation was important.

DISCUSSION

Cytolytic T cells may be stimulated through their T-cell receptor to become lytic, not only by the appropriate antigen–MHC complex, but also by antibodies to the T-cell receptor or CD3 complex. Previous experiments reported involve anti-T-cell receptor anti-CD3 antibodies binding to Fc receptor-bearing target cells (Kranz *et al.*, 1984; Mentzer *et al.*, 1985; Staerz & Bevan, 1985; Yssel *et al.*, 1986; Van Seventer *et al.*, 1987). Hybrid monoclonals, cross-linking T-cell receptors and tumour cells have also been used (Staerz & Bevan, 1985; Perez *et al.*, 1985; Jung *et al.*, 1987; Lanzavecchia & Scheidegger, 1987). In the experiment reported here we found that an antibody to the T-cell receptor, Mx9, could cross-link CD8 and CD4 clones and cause the selective lysis of the latter. Unlike previous studies, which showed that intact IgG and Fc receptors were essential, F(ab′)$_2$ Mx9 was able to induce lysis effectively. This is because the F(ab′)$_2$ fragment is still capable of cross-linking the cells used, as each reacts with Mx9. The F(ab′)$_2$ preparation was not significantly contaminated with IgG as revealed by SDS–PAGE, and more relevantly by the inability of Mx9 F(ab′)$_2$ to induce lysis of an EBV line, in contrast to the Mx9 IgG (Fig. 7). Stimulus through the TcR or the CD3 complex was described to be essential for inducing lytic action (e.g. Mentzer *et al.*, 1985).

We report that the cross-linking by anti-class I antibodies (which recognize antigens present on high density on both effector and target cells) did not activate the lytic mechanism.

In our experiments the cytotoxicity was relatively unidirectional. Only CD8 clones exerted a strong cytolytic action. This may appear to contradict the results of other authors (Mentzer *et al.*, 1985; Blanchard *et al.*, 1987) who have shown lysis by CD4 cells. These results may be reconciled if the frequency of lytic CD4 cells is low, in the region < 5%, as we tested 19 clones only. The lytic capacity of CD8 cells and their relative resistance to lysis (c.f. CD4) can explain why cell lines maintained in long-term culture in the presence of the anti-TcR antibodies show predominantly, if not exclusively, a CD8 phenotype, as described by Boylston *et al.* (1985). In this study CD8 clones were also cytotoxic against other CD8 clones, but the degree of ^{51}Cr released was less. This suggest that the CD8 clones possess some protective mechanism against the cytolitic hit. Therefore, it would be interesting to investigate the production of lymphokines by both the types of clones after stimulation with specific antibody. It was reported that transforming growth factor beta, TGFβ, inhibits lytic activity (Rook *et al.*, 1986), and this may be part of the protective mechanism.

The results obtained here have indicated that anti-receptor antibody may have profound effects on the cells that it recognizes. The activation of the lytic process enables CD8 cells to destroy closely related CD4 cells as shown here, and in principle idiotypically related B cells also. This would provide a mechanism by which anti-idiotypic antibodies could limit the growth of T-cell clones. There are examples in which idiotypic antibodies can profoundly influence the immune system on a long-term basis (reviewed in Bluestone *et al.*, 1986). The mechanism by which this effect is mediated has not been elucidated, and it is apparent that antibodies reacting with the cell surface need not in itself be deleterious. However, by focusing activated cytolytic T cells to the cell surface of T, especially CD4 cells, as shown here, or B cells, anti-idiotypic antibodies may induce lysis of these cells, with long-term immunological consequences. Since anti-idiotypic antibodies, unlike anti-CD3 antibodies, are induced *in vivo*, this study indicates that this form of immune regulation involving anti-T-cell receptor antibodies is possible, and conceivably may be of importance *in vivo*.

ACKNOWLEDGMENTS

We thank Miss G. Portillo for assistance with preparation and purification of F(ab')$_2$ fragments and Dr A. Boylston, St Mary's Hospital, London for gift of 42/ICI, Dr M. Kahan for assistance with cell separation in the FACStar, Dr M. Contreras and her colleagues at National Blood Transfusion Centre, Edgware, Middlesex for supplies of blood and Dr Junji Hamura of Ajinomoto for supplies of purified human recombinant IL-2.

This work was supported by the Wellcome trust and 'Cenci-Bolognetti' Foundation grant. Dr M. Londei is currently supported by the Juvenile Diabetes Foundation International.

REFERENCES

BINZ H. & WIGZELL H. (1975) Shared idiotypic determinants on B-lymphocytes and T-lymphocytes reactive against same antigenic determinants. Demonstration of similar or identical idiotypes on IgG molecules and T cell receptors with specificity for the same allo-antigens. *J. exp. Med.* **142,** 197.

BLANCHARD D., VAN ELS C., BORST J., CARREL S., BOYLSTON A., DE VRIES J.E. & SPITS H. (1987) The role of the T cell receptor, CD8 and LFA-1 in different stages of the cytolytic reaction mediated by alloreactive T lymphocytes clones. *J. Immunol.* **138,** 2417.

BLUESTONE J.A., LEO O., EPSTEIN S.L. & SACHS D.H. (1986) Idiotypic manipulation of immune response to transplantation antigens. *Immunol. Rev.* **90,** 5.

BOYLSTON A. & COSFORD P. (1985) Growth of normal human T lymphocytes induced by monoclonal antibody to the T cell antigen receptor. *Eur. J. Immunol.* **15,** 738.

BURDETTE S. & SCHWARTZ R.S. (1987) Idiotypes and idiotypic networks. *New Engl. J. Med.* **317,** 219.

CARREL S., ISLER P., SCHREYER M., VACCA A., SALVI S., GIUFFRE L. & MACH J.P. (1986) Expression on human thymocytes of the idiotypic structures (Ti) from two leukemia T cell lines Jurkat and HPB-ALL. *Eur. J. Immunol.* **16,** 649.

EICHMANN K. & RAJEWSKY K. (1975) Induction of T and B cell immunity by anti-idiotypic antibody. *Eur. J. Immunol.* **5,** 661.

JUNG G., LEDBETTER J.A. & MULLER-EBERHARD J. (1987) Induction of cytotoxicity in resting human T lymphocytes bound to tumor cells by antibody heteroconjugates. *Proc. natl. Acad. Sci. U.S.A.* **84,** 4661.

KRANZ D.M., TONEGAWA S. & EISEN H.N. (1984) Attachment of an anti-receptor antibody to non target cells renders them susceptible to lysis by a clone of cytotoxic T lymphocytes. *Proc. natl. Acad. Sci. U.S.A.* **81,** 7922.

LANZAVECCHIA A. & SCHEIDEGGER D. (1987) The use of hybrid hybridomas to target human cytotoxic T lymphocytes. *Eur. J. Immunol.* **17,** 105.

LONDEI M., GRUBECK-LOEBENSTEIN B., DE BERARDINIS P., GRENALL C. & FELDMANN M. (1988) Efficient propagation and cloning of human T cells in the absence of antigen using OKT3, IL-2 and antigen presenting cells. *Scand. J. Immunol.* **27,** 35.

MENTZER S.J., BARBOSA J.A. & BURAKOFF S.J. (1985) T3 monoclonal antibody activation of non specific cytolysis: A mechanism of CTL inhibition. *J. Immunol.* **135,** 34.

MILLER G., SHOPE T., LISCO D., STITT D. & LIPMAN M. (1972) Epstein-Barr virus: transformation, cytopatic changes and viral antigens in squirrel monkey and marmoset leukocytes. *Proc. natl. Acad. Sci. U.S.A.* **69,** 383.

PEREZ P., HOFFMAN R.W., SHAW S., BLUESTONE J.A. & SEGAL J. (1985) Specific targeting of cytotoxic T cells by anti-T3 linked to anti-target cell antibody. *Nature (Lond.)*, **316,** 354.

ROOK A.H., KEHRL J.H., WAKEFIELD L.M., ROBERTS A.B., SPORN M.B., BURLINGTON D.B., LANE H.L. & FAUCI A.S. (1986) Effects of transforming growth factor β on the functions of natural killer cells: depressed cytolytic activity and blunting of interferon responsiveness. *J. Immunol.* **136,** 3916.

STAERZ U.D. & BEVAN M.J. (1985) Cytotoxic T lymphocyte-mediated lysis via the Fc receptor of target cells. *Eur. J. Immunol.* **15,** 1172.

STAERZ U.D., KANAGAWA O. & BEVAN M.J. (1985) Hybrid antibodies can target sites for attack by T cells. *Nature (Lond.)*, **314,** 628.

STRAYER D.S., COSENZA M., LEE W., ROWLEY D.A. & KÖHLER M. (1974) Neonatal tolerance induced by antibody against antigen specific receptors. *Science*, 186, 640.

Van Seventer G.A., Kuijpers K.C., Van Lier R.A.W., De Groot E.R., Aarden L.A. & Melief C.J.M. (1987) Mechanism of inhibition and induction of cytolytic activity in cytotoxic T lymphocytes by CD3 monoclonal antibodies. *J. Immunol.* **139,** 2545.

YSSEL H., BLANCHARD D., BOYLSTON A., DE VRIES J.E. & SPITS H. (1986) T cell clones which share T cell receptor epitopes differ in phenotype, function and specificity. *Eur. J. Immunol.* **10,** 1187.