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Interactions of human T cell subsets during the induction of cytotoxic T lymphocytes: the role of interleukins

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SUMMARY

In this work we study the role of subsets of human T cells, detectable by the OKT series of monoclonal antibodies, in the production of and the response to the lymphokine interleukin-2 (Il-2) during the course of an allogeneic cytotoxic T lymphocyte response *in vitro*. The results obtained establish that the Il-2 producer cells reside within the OKT4 positive T cell subset. Once produced, Il-2 mediates the clonal expansion of alloantigen-activated cytotoxic T killer cells which reside in the OKT8 positive T cell subset. Il-2 appears to have no mitogenic activity on the activated OKT4 positive T cells which produce the lymphokine. In order to release Il-2, the OKT4 positive T cell requires a stimulus, such as allogeneic cells or the lectin phytohaemagglutinin A (PHA). Macrophages are also required for Il-2 production, but the macrophage requirement can be bypassed by a soluble macrophage product as found in supernatants of lymphocyte cultures stimulated with lipopolysaccharide (LPS), the biological activity presumably representing Interleukin-1 (Il-1).

INTRODUCTION

The dissection of cellular interactions during the course of the activations of murine cytotoxic T lymphocytes (CTL) has been facilitated by the availability of serological reagents that distinguish between functionally different subsets of T cells (Cantor & Boyse, 1975). It is apparent that T lymphocytes with the Lyt 1 marker comprise the T helper lymphocytes, while the Lyt 23 positive T cell subset includes the CTL precursors (CTL-P) (Cantor & Boyse, 1975; Wagner & Röllinghoff, 1978). Furthermore, antigen- or mitogen-activated Lyt 1^+ T helper cells release a soluble product, Interleukin-2 (Il-2), which acts as a non-specific and H-2 non-restricted 'second' signal during the course of antigen-specific CTL-P.

In humans, these cellular events have not yet been elucidated in detail, but with the recent availability of the OKT series of monoclonal antibodies the dissection of such interactions becomes possible. Thus, a number of studies, performed in different laboratories, indicate that the OKT8 positive human T cell subset includes hapten- or influenza virus-reactive (Friedman *et al.*, 1979, 1981; Biddison, Sharrow & Shearer, 1981) and alloreactive T pre-killer cells (Reinherz *et al.*, 1980)

Abbreviations: PHA = Phytohaemagglutinin A; LPS = Lipopolysaccharide; Il-1 = Interleukin-1; Il-2 = Interleukin-2; CTL-(P) = cytotoxic T lymphocyte (precursor); FCS = fetal calf serum; PBMNC = peripheral blood mononuclear cells; SRBC = sheep red blood cells; smIg = surface membrane immunoglobulin; FITC = fluorescein isothiocyanate; C = complement; MLC = mixed lymphocyte culture; LAF = lymphocyte activating factor.

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while the OKT4 positive subset contains T cells with helper function for B and T cell differentiation (Thomas *et al.*, 1981; Kung *et al.*, 1979). Moreover, the OKT4 positive and the OKT8 positive T cell subsets were reported to be mutually exclusive and to comprise the entire T cell population (Kung *et al.*, 1979).

In the present communication we analyse the role of subsets of T cells detectable by the OKT series of antisera in the production of interleukins as well as in the induction of human CTL responses *in vitro*. Our studies clearly show that the cellular interactions required for successful human CTL responses closely parallel the series of events that currently are considered to occur in the murine system. Thus, one subset of T cells within the OKT4 positive T cells produces Il-2, whereas the OKT8 positive T cell subset includes the precursors of alloreactive CTL responding to Il-2 after its previous interaction with antigen or mitogen. As in the murine system, the productior. of Il-2 by OKT4 positive T cells requires the presence of antigen or mitogen and macrophages. However, the need for macrophages can be replaced by a soluble product from macrophage cultures stimulated with lipopolysaccharide (LPS). This product presumably represents Interleukin-1.

MATERIALS AND METHODS

Medium. In all experiments the medium used was RPMI 1640 (Seromed, Munich, FRG) supplemented with penicillin (100 units/ml), streptomycin (100 μg/ml), L-glutamine (2 mM, Seromed, Munich, FRG), HEPES buffer (10 mM; GIBCO, Glasgow, Scotland), sodium bicarbonate (0·15%, GIBCO, Glasgow, Scotland), and fetal calf serum (FCS) (1–10% [v/v], Seromed, Munich, FRG).

Cell separation. Fresh peripheral blood mononuclear cells (PBMNC) were obtained from consenting healthy volunteers by Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) density gradient centrifugation. Highly enriched T cell populations were obtained by dual passage of $50–100 \times 10^6$ PBMNC over nylon wool columns, according to Julius, Simpson & Herzenberg (1973). Subsequently the effluent cells (approximately 30–40% of the input number) were layered onto the surface of a 90 mm glass petri dish for 4 hr at 37°C in a humidified atmosphere with 5% CO_2 to remove adherent cells. More than 95% of the non-adherent cells formed rosettes with neuraminidase (Behringwerke, Marburg, FRG) treated sheep red blood cells (SRBC/BAG, Lich, FRG) and less than 1% of the cells had surface membrane immunoglobulin (SmIg) detectable by direct immunofluorescence using fluorescein isothiocyanate (FITC)-labelled goat anti-human immunoglobulin (Meloy, Springfield, USA). The T cells were further subdivided into $OKT4^+$ and $OKT8^+$ subsets by negative selection procedures. Briefly, 10×10^6/ml T cells were incubated with 5% (v/v) of monoclonal mouse anti-human OKT4 or OKT8 antibodies (Ortho Pharmaceutical Co., Raritan, New Jersey, USA) respectively, for 30 min at 4°C. OKT4 antibody identifies an antigen on the human helper subclass of lymphocytes, which comprise about 60% of all peripheral T lymphocytes; OKT8 antibody is specific for the suppressor and cytotoxic T cell subset, that constitutes about 40% of the T cells in peripheral blood (Thomas *et al.*, 1981; Kung *et al.*, 1979). After 30 min, fresh rabbit serum, as a source of complement, was added at a final dilution of 1 : 12. This mixture was incubated for 45 min at 37°C in a water bath and then washed three times in culture medium.

To test for the effectiveness of the procedure, 100 μl of the cells (1×10^6) that had been treated with OKT4 or OKT8 antibody and complement were incubated with 5 μl of OKT antibody and OKT8 antibody respectively for 30 min at 4°C. Binding of the antibodies to the cells was visualized by indirect immunofluorescence using fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse $F(ab)_2$ fragments (Meloy, Springfield, Virginia, USA) at a final dilution of 1 : 10 for 45 min at 4°C. Usually, treatment of T cells with OKT4 antibody plus complement resulted in killing of 60–70% of the total number of T cells, 94–98% of the remaining viable cells stained with OKT8 antibody less than 2% with OKT4 antibody, 0% were SmIg positive. Less than 1% of the cells showed non-specific esterase activity, a marker for macrophages as determined by the method of Tucker, Pierre & Jordon (1977). Accordingly, this population is referred to as the $OKT8^+$ T cell subset.

Treatment of T cells with OKT8 antibody plus complement resulted in killing of 30–40% of the

total number of T cells. Ninety-five percent of the remaining viable cells stained by indirect immunofluorescence with OKT4 antibody, and about 2% with OKT8 antibody. None of the cells were SmIg positive, and less than 1% showed non-specific esterase activity. Accordingly, this cell population is referred to as $OKT4^+$ T cell subset.

Macrophages were obtained by incubating $50–70 \times 10^6$ freshly drawn PBMNC in 90 mm glass petri dishes at 37°C in a 5% CO_2 atmosphere overnight in 10 ml RPMI 1640 medium, containing 10% FCS. Non-adherent cells then were removed by vigorous washing of the petri dishes with a Pasteur pipette. The adherent cells (between 5% and 15% of the input cells) were then removed mechanically with a rubber policeman. In some experiments, the adherence procedure was performed twice. More than 96% of the adherent cells showed non-specific esterase activity. This cell population is referred to as macrophages.

Interleukin-1 (Il-1) production. The biological activity designated as Interleukin-1 (Il-1) was produced by incubating 1×10^6/ml PBMNC with 20 μg/ml lipopolysaccharide (LPS) from *E. coli* (strain 027:B8) (DIFCO Laboratories, Detroit, Michigan, USA) for 24 hr at 37°C in humidified atmosphere of 5% CO_2. The cell free supernatant was harvested, filtered through a 0·22 μm filter (Millipore, Molsheim, France) and stored at −20°C until use. These supernatants did not have Interleukin-2 (Il-2) activity (detected as described below).

Assay for Il-1. Operationally, in the mouse system, Il-1 has been defined by its capacity to allow antigen- or mitogen-activated T helper cells to produce Il-2 (Smith, Gilbride & Favata, 1980a; Smith *et al.*, 1980b). Accordingly, 1×10^6/ml human T cells were incubated in the presence of 1 μg/ml PHA and serial dilutions of LPS-induced supernatants for 24 hr in 2 ml Linbro wells (RPMI 1640, 5% FCS, 37°C, 5% CO_2). Subsequently, the resulting supernatants were harvested and tested for Il-2, the activity of which is directly correlated to the amount of Il-1 activity in the LPS supernatant. In addition to the method described here, in some experiments, Il-1 activity was measured by employing the traditionally used assay for Il-1, i.e., to test for the mitogenic effect on murine thymocytes in the presence of submitogenic doses of PHA as detected by titrated thymidine incorporation (Smith *et al.*, 1980a, 1980b).

Interleukin-2 (Il-2) production. Supernatants rich in Il-2 activity were produced by two methods. First, $1·5 \times 10^6$/ml PBMNC of donor A were co-cultured in 2 ml RPMI 1640 medium containing 5% FCS in a Linbro culture plate (Linbro FB-24 Tc, Linbro Chem. Corp., Hamden, Conn., USA) for 7 to 10 days with $2·5 \times 10^6$/ml X-irradiated (3,000 rad, Philips machine RT 200, Müller, Hamburg, FRG) PMNC from donor B (B_x cells). Then $0·5 \times 10^6$/ml responder cells were restimulated in a secondary MLC (2° MLC) with $2·5 \times 10^6$/ml B_x cells. The cell culture supernatant of the 2° MLC was then harvested between 24 and 72 hr after restimulation, filtered and stored at −20°C until use.

The second method to produce supernatants rich in Il-2 activity was to stimulate cells with phytohemagglutinin (PHA). Accordingly, 1×10^6/ml T cells were treated with either OKT4 or OKT8 monoclonal antibodies (5% v/v) plus rabbit complement (final dilution 1:12) or with complement alone in a total volume of 100 μl. Subsequently, the surviving cells were washed twice in culture medium and stimulated in the presence or absence of 1×10^5/ml macrophages with 2 μg PHA in a total volume of 2 ml in Linbro FB-24 Tc tissue culture plates in RPMI 1640, 5% FCS at 37°C, in a humidified 5% CO_2 atmosphere. In previous experiments it was shown that a 5 hr exposure time to PHA was sufficient to induce Il-2 production. Accordingly, in order to remove as much PHA as possible, the cell culture medium containing PHA was replaced after 5 hr by fresh medium without PHA and the cells were incubated for a further 36 hr. Thereafter, cell free culture supernatants were harvested, filtered, and stored at −20°C until use.

Assessment of Il-2 activity. Il-2 activity of cell free culture supernatants was assayed by testing its capacity to sustain the proliferation and specific cytolytic activity of alloantigen-activated secondary cytotoxic T lymphocytes (CTL). Accordingly, $1·5 \times 10^6$/ml PBMNC derived from donor A were co-cultured with $2·5 \times 10^6$/ml X-irradiated (3,000 rad) allogeneic PBMNC of an allogeneic donor B (= B_x cells) in 2 ml culture wells for 7 days (37°C, 5% CO_2) (1° MLC). Half a million responding cells per ml were restimulated with $2·5 \times 10^6$/ml B_x cells for 4 days (2° MLC). Dead cells (mostly B_x) were removed by Ficoll-Hypaque centrifugation. Fifty thousand viable cells were then seeded in 200 μl culture medium in flat bottom microtitre plates (Greiner, Nürtingen, FRG) in the

presence of serial dilutions of the Il-2 rich supernatants to be tested. These cultures were assayed after 48 hr for cell proliferation (^{3}H-thymidine incorporation), or, after 96 hr for their capacity to lyse 1×10^3 B target cell blasts, which had been activated by PHA 72 hr previously and labelled with $Na_2{}^{51}CrO_4$ (New England Nuclear, Dreieich, FRG) for 2 hr before the assay.

Tritiated thymidine incorporation was measured as follows: cell cultures were pulsed with 1 μCi/well ^{3}H-thymidine (New England Nuclear, Dreieich) for 4 hr, then harvested on filters using a Skatron multiwell harvester and ^{3}H-thymidine incorporation counted in a liquid scintillation β-counter (Packard Instruments, Frankfurt, FRG).

Lytic activity of the 2°MLC-derived, Il-2-dependent CTL was tested as described (Wagner & Röllinghoff, 1978). Briefly, the cells of one culture were incubated for 3 hr with $Na_2{}^{51}CrO_4$ labelled B target cell blasts. Thereafter the cells were centrifuged (500 *g*, 5 min) and the radioactivity in the cell pellet and in the supernatant was determined separately in a Packard Gamma-counter (Packard Instruments, Frankfurt, FRG). Percentage specific lysis was calculated according to the formula described previously (Wagner & Röllinghoff, 1978). Since the specific lytic activity of the CTL correlates directly with the activity of Il-2 present in the supernatant to be tested, the assay can be standardized by comparing the activity of the test sample with the activity of standard preparation of Il-2 (pooled supernatants from seven different PHA-stimulated PBMNC cultures). Thus, specific CTL-mediated lysis was converted into Il-2 activity by using the formula:

$$\text{Il-2 activity in a given sample} = \frac{\%\text{ specific lysis of target cells obtained with a given concentration of the Il-2 to be tested}}{\%\text{ specific lysis of target cells obtained with the same concentration of a standardized Il-2 preparation}}$$

The relative Il-2 titres obtained in this assay system correlated well (correlation coefficient $r = 0{\cdot}98$) to titres obtained when the supernatants were assayed for their capacity to sustain the proliferation of both, a murine Il-2 dependent cell line (kind gift of Dr Th. Hünig, University Würzburg, FRG) and a human Il-2 dependent T cell line, which was kept in culture for more than 8 weeks (unpublished data).

RESULTS

Cellular requirements for the induction of Il-2 production

Il-2 has been defined as a T helper cell product able to induce clonal expansion of antigen- or mitogen-activated CTL-P *in vitro* (Wagner & Röllinghoff, 1978; Smith *et al.*, 1980a, 1980b; Wagner *et al.*, 1980). Thus Il-2 should be detectable in supernatants of an allogeneic MLC, in the course of which T helper cells become activated. That Il-2 production occurs during the MLC is shown in experiments described in Table 1. PBMNC ($1{\cdot}5 \times 10^6$/ml) from donor A were stimulated with

Table 1. Il-2 production during the course of a 2° MLC

1° MLC	Restimulation (after 7 days) with 2° MLC	Il-2 activity in cell free supernatant after restimulation (hr) 24	48	72
A anti-B_x	A_x	5	0	0
	B_x	39	55	11

For details of the cultures see Materials and Methods section.

X-irradiated PBMNC ($2{\cdot}5 \times 10^6$/ml) from an allogeneic donor B. After 10 days the responding cells ($0{\cdot}5 \times 10^6$/ml) were restimulated with X-irradiated PBMNC from either donor A or B ($2{\cdot}5 \times 10^6$/ml) (A_x or B_x cells). Between 24 and 72 hr after restimulation, cell culture supernatants were harvested and assayed for Il-2 activity. As can be seen (Table 1), in the allogeneic A anti-B_x combination the maximum Il-2 release occurred 48 hr after restimulation, thereafter it declined rapidly. No Il-2 release could be detected if the primary culture was restimulated with cells autologous to the responding cell type (A anti-A_x).

Subsequently, experiments were designed to identify the cells necessary for the production of Il-2 in response to the lectin phytohaemagglutinin A (PHA). Thus, T cells (1×10^6/ml) were treated with either OKT4 antibody or OKT8 antibody (5% v/v) plus complement (C) or complement alone. Subsequently, the remaining viable cells (about 40% (range 25–45%) of the input cells after treatment with OKT4 antibody and about 60% (range 50–80%) after treatment with OKT8 antibody) were stimulated in the presence or absence of 1×10^5/ml macrophages with 1 μg/ml PHA for 5 hr. Unbound PHA was washed off the culture and 36 hr later the culture supernatants were harvested and tested for Il-2 activity. Whereas none of the purified T cell populations produced significant amounts of Il-2, if supplemented with macrophages, high Il-2 activity was detectable both in the supernatants of T cells treated with C alone and of T cells treated with OKT8 antibody plus C. In contrast, supernatants of T cells treated with OKT4 antibody plus C contained almost no Il-2 (Table 2).

Table 2. Il-2 production by PHA-activated T cells

Supernatant to be tested for Il-2 activity derived from PHA stimulated	Il-2 activity in 36 hr supernatant
(1) T cells treated with C alone	18
T cells treated with C plus macrophages	104
(2) T cells treated with OKT8 antibody plus C	12
T cells treated with OKT8 antibody plus C plus macrophages	112
(3) T cells treated with OKT4 antibody plus C	5
T cells treated with OKT4 antibody plus C plus macrophages	8
Macrophages alone	5

These results clearly indicate that the T cells which are involved in Il-2 production reside within the OKT4 positive T cell population. In addition they show that the activation of the OKT4 positive T cells to produce Il-2 occurs only in the presence of macrophages. Moreover, it can be concluded that within the untreated T cells the Il-2 producer cells are solely recruited from the OKT4 positive T cell subset, as the Il-2 activity is comparably high in both groups (Table 2; 1. and 2.). Finally, it follows that OKT8 positive cytotoxic suppressor T cells do not seem to affect Il-2 production in the OKT4 positive T cell population.

Target cell for the T helper cell product Il-2

In order to establish which T cell subset forms the target cell for the action of Il-2, allogeneic MLC were set up. Seven days after the onset of the cultures, responder cells were stimulated with the respective allogeneic stimulator cells (2° MLC) and 4 days after restimulation, 5×10^5 responder cells were treated with either OKT4 antibody plus C, OKT8 antibody plus C or with C alone. After washing twice, 5×10^4 of the remaining viable cells were incubated in a volume of 2 ml in the presence of 50% (v/v) Il-2 containing supernatants derived from PHA-stimulated OKT4 positive T cells. Subsequently, the cells were tested for their proliferative response (after 72 hr) as well as for their cytotoxic activity against the relevant target cells (after 96 hr). It is clearly apparent from the results in Table 3, that the alloantigen-activated OKT8 positive T cell subset is the one which

Table 3. The target cell of Il-2 derived from OKT4-positive T cells

2° MLC generated in the allogeneic combination	Responding T cell subset tested	Cell proliferation of the responding cells after incubation with OKT4+ T cell derived supernatant (c.p.m. ± s.d.)*	Cytotoxic activity of the responding cells after incubation with OKT4+ T cell derived supernatant (% specific lysis)†
	OKT4 positive (treated with OKT8 antibody plus C)	2,500 ± 800	10 ± 8‡
A anti-B_x	OKT8 positive (treated with OKT4 antibody plus C)	22,700 ± 1,900	66 ± 12
	unseparated (treated with C alone)	25,000 ± 1,500	64 ± 15
	OKT4 positive	1,350 ± 380	0 ± 2
B anti-C_x	OKT8 positive	45,500 ± 4,000	55 ± 5
	unseparated	39,000 ± 5,500	61 ± 11
	OKT4 positive	3,200 ± 1,000	12 ± 9
C anti-D_x	OKT8 positive	40,000 ± 5,000	66 ± 18
	unseparated	44,000 ± 4,000	65 ± 9

* counts per minute ± standard deviation of six replicates.

† effector to target cell ratio = 50:1, assay time 3 hr.

‡ mean ± standard deviation from three independent determinations.

Cells incubated without Il-2 supernatants were dead within 24 hours after onset of the test culture. Thymidine incorporation of these cells was 880 ± 480 c.p.m., specific cytotoxic activity was 3% ± 3%.

responded to Il-2 proliferation as well as by an increase in cytotoxic activity. In contrast, activated OKT4 positive T cells proliferated only marginally in response to Il-2 and mounted very low cytotoxic activity. This conclusion is further substantiated by the observation, that the OKT8 positive T cells (responding T cells) incorporated as much radioactive thymidine, and mounted about the same cytotoxic activity as the non-depleted T cells.

These data indicate that Il-2 functions a physiological mitogen for alloantigen-activated OKT8 positive T cells which include the cytotoxic T killer cells.

Il-1 substitutes for the macrophage requirement during the activation of the OKT4 positive Il-2 producer cells

Several laboratories have shown that macrophages are required for the induction of a variety of T lymphocyte effector functions, including their capacity to produce Il-2 (Rosenstreich, Farrar & Dougherty, 1976; Habu & Raff, 1977; Rosenstreich & Mizel, 1978; Larsson, Coutinho & Martinez, 1980; Todd, Reinherz & Schlossman, 1980; Weinberger *et al.*, 1981; Sredni *et al.*, 1981; Oppenheim *et al.*, 1980; Maizel *et al.*, 1981). Furthermore, macrophages activated with LPS are known to release Interleukin-1 (Il-1, formerly designated 'lymphocyte activating factor' LAF). One of the biological activities of Il-1 is to substitute for the macrophage requirement in mitogen-driven proliferative T cell responses (Rosenstreich & Mizel, 1978; Larsson *et al.*, 1980; Todd *et al.*, 1980; Weinberger *et al.*, 1981; Maizel *et al.*, 1981). However, it is not clear, whether supernatants containing Il-1 as assayed in terms of proliferation of the thymic target cells can effectively substitute for Il-2 production by T cells in humans.

Experiments designed to address this question establish that upon activation of macrophages by LPS highly active Il-1 was released into the culture supernatant (Table 4). The Il-1 activity of a given preparation was assayed independently in two different systems. Firstly, the supernatants, containing Il-1, were shown to enhance mitogenesis of PHA-activated murine thymocytes in a

Table 4. Enhancement of murine thymocyte mitogenesis and facilitation of Il-2 production by LPS-induced Il-1 containing cell culture supernatants

Murine thymocytes (1×10^5/ml) incubated with			T cells (1×10^6/ml) incubated with		
LPS-induced supernatants (concentration (% v/v))	PHA (0·5 μg/ml)	^{3}H-thymidine incorporation (c.p.m. ± s.d.)*	LPS-induced supernatants (concentration (% v/v))	PHA (1 μg/ml)	Il-2 activity in the culture supernatant
50	no	650 ± 80	50	no	0
12	no	830 ± 85	12	no	5
6	no	750 ± 115	6	no	2
50	yes	50,325 ± 3,100	50	yes	60
25	yes	35,255 ± 5,200	25	yes	55
12	yes	18,800 ± 1,360	12	yes	37
6	yes	4,350 ± 1,100	6	yes	20
0	yes	1,800 ± 280	0	yes	15

* Counts per minute ± standard deviation of six replicates.

dose-dependent manner (Table 4). Secondly, the same culture supernatants substituted effectively in a dose–response manner (Table 4) for the macrophage requirement during the PHA-mediated activation of Il-2 producer T cells.

DISCUSSION

The aim of this work was to identify the human T cell subsets that are activated to produce Il-2 by the T cell mitogen PHA as well as to determine the subset of T cells that respond *in vitro* to Il-2 and become cytotoxic T cells (CTL). Furthermore, we wished to understand the role of macrophages and their soluble product Il-1 during the activation phase of Il-2 producer T cells.

The results obtained establish that the Il-2 producer cells reside within the OKT4 positive T cell subset, whereas the OKT8 positive T cells are those that respond to Il-2 by proliferation and cytotoxicity. Moreover, Il-2 production only occurs, if macrophages, or their soluble product Il-1, are additionally present.

Most of our ideas on how T cells and macrophages interact in cell-mediated immune responses come from work in murine systems. Here, the Il-2 producer T cells have been identified as Lyt 1^+ cells that function as helper cells (Wagner & Röllinghoff, 1978). The target cell for Il-2 is either within the antigen activated Lyt 123^+ or the Lyt 23^+ T cell subset. Both subsets respond to the Il-2 stimulus by clonal expansion and differentiation into CTL (Wagner & Röllinghoff, 1978; Wagner *et al.*, 1980).

Our studies using recently available monoclonal antibodies to dissect human T cell subsets involved in cell-mediated immunity *in vitro*, indicate a close parallel with events considered to occur during murine responses. Thus, the OKT4 positive subset appears to be equivalent to murine Lyt 1 positive T cells in that the OKT4 positive subset is the one which upon mitogen stimulation produces Il-2. As in the mouse, the production of Il-2 requires that macrophages are also present during the mitogen stimulation of OKT4 positive cells. However, the role that macrophages play is not simply one of passive mitogen presentation to the T cells since their function can be substituted by Il-1, a soluble product released from macrophages following stimulation with LPS (Table 4). Thus, we can assume that, at least during the lectin response of OKT4 positive T cells, the function of macrophages is to supply a factor, Il-1, that conditions the OKT4 positive T cell to respond and to produce Il-2. In line with this conclusion are our own data (unpublished), and the observation of others (Weinberger *et al.*, 1981), that if macrophages are treated with agencies such as

glutaraldehyde or u.v. light, which prevent their secretory functions, then macrophages fail to subserve an accessory role.

It is now well accepted that the Lyt 1 positive T cell subset of the mouse subserves a helper cell function in both B and T cell responses. How exactly helper cells effect their function is still ill defined, but we (Wagner *et al.*, 1980), as well as others (for review see Möller, 1980) have argued that it is Il-2 that functions as a helper cell product to a second subset of T cells, i.e. the CTL precursors. Our present studies demonstrate that, as in the mouse, the human T cell subset responding to Il-2 is distinguishable from that which produces Il-2. Thus, it is the OKT8 positive T cell that responds to Il-2, although such responses only occur following an initial interaction with a primary stimulus which in the present study was an alloantigen. Although not formally proven, it has been suggested that antigen instead of a mitogen subserves a similar primary function driving Il-2 responsive cells to express receptors for Il-2 (Larsson, 1981). Subsequently, the cells proliferate following this interaction with Il-2 and differentiate into CTL. Our results obtained in the human system are compatible with this series of events with the OKT4 T cell subset producing Il-2 and the OKT8 subset responding to Il-2 to become cytotoxic.

Based on data obtained in the mouse system (Glasebrook *et al.*, 1981), the question arises, whether or not the OKT4 positive Il-2 producer cell can use its own product as growth factor. The data obtained here indicate that this type of cell does not respond to Il-2. This is in agreement with work from Larsson & Coutinho (personal communication) who claim that stimulated murine Lyt 1 positive T cells produce Il-2 but do not respond to Il-2. On the other hand, Schreier *et al.* (1980) established clones of murine T cell origin, which on the one hand produced Il-2, and on the other hand needed Il-2 as an essential component for their continuous proliferation. Thus, the question is open, whether the latter finding represents a unique feature of these particular clones or whether that observation can be generalized.

Observations of *in vitro* mechanisms of cell-mediated immunity are likely to enhance our understanding of the pathophysiology and control of certain immunological diseases. For example, blood T cells from patients with chronic lymphocytic leukaemia (CLL) were reported to have T helper cell defects in their capacity to help allogeneic B cells to secrete specific antibody (Chiorazzi *et al.*, 1979). We have extended these studies by showing that virtually no PHA-mediated Il-2 production occurred from OKT4 T cells from four patients with CLL of the T cell type (Solbach *et al.*, 1981).

On the other hand it is interesting to note that in patients with Sézary's syndrome, a cutaneous lymphoma, high numbers of malignant proliferating cells have OKT4 surface phenotype (Haynes *et al.*, 1981). At least in a proportion of these patients the malignant proliferating cells produce high amounts of Il-2 upon stimulation with PHA (Solbach *et al.*, 1980).

Moreover, there is evidence that certain cell lines derived from human cutaneous T cell lymphomas not only produce, but also respond to Il-2 (Gootenberg *et al.*, 1981). Whether such 'autocrine secretion', a term proposed by Sporn & Todaro (1980) for this type of self-stimulation plays an aetiological role in the growth properties of T cell malignancies needs to be further investigated.

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