Immunology 1982 **47** 633

Variation in accessory cell requirements in human mixed lymphocyte response to leukaemic cell lines

DENISE O'KEEFE & LEONIE ASHMAN *Department of Microbiology and Immunology, The University of Adelaide, Adelaide, South Australia, Australia*

Accepted for publication 25 May 1982

Summary. Human leukaemia and lymphoma cell lines were investigated as stimulating cells in an allogeneic mixed lymphocyte response. Purified T cells and unfractionated mononuclear cells from normal donors were used as responders. The cell lines fell into three groups: (i) those which stimulated allogeneic responder T cells in the presence or absence of accessory (non-T) cells; (ii) those which stimulated T cells only in the presence of accessory cells; and (iii) those which failed to stimulate in either case. The accessory function was provided by adherent cells and non-adherent, non-T cells. There was no correlation between the stimulatory capacity of these cell lines and the presence of serologically defined HLA-DR determinants. These results are discussed in the context of the current two signal hypothesis for T-cell activation.

INTRODUCTION

The mixed lymphocyte reaction (MLR) has been used extensively in research as an *in vitro* assay for definition and characterization of the gene products of the major histocompatibility complex in murine and human systems (Meo, David & Shreffler, 1976; Dausset & Fradelizi, 1977). The clinical applications of the MLR are primarily as an *in vitro* correlate of cell-mediated immune function (Wolos & Davey, 1980) and in fields such as bone marrow transplantation, as a final test for tissue compatibility.

The MLR is generally interpreted as a proliferative response by T cells to determinants present on allogeneic stimulating cells. Adherent cells or monocytes have frequently been reported as necessary accessory cells in the response (Greineder, Shevach & Rosenthal, 1976, Huber, Fink, Leibold, Schmalzl, Peterson, Klareskog & Braunsteiner, 1981). While there is little argument on the nature of the responding cell, the nature and role of the stimulating cell is unclear. In the mouse system, not all cell types can stimulate; in particular non-lymphoid cells such as cultured fibroblasts or epithelial tumour cells fail to stimulate allogeneic lymphocytes (Talmage, Woolnough, Hemmingsen, Lopez & Lafferty, 1977). Some correlation has been found between the ability of cells to stimulate and the expression of H2-I region determinants (Niederhieber, 1978), however Ia negative cells such as the mesenchymal line, P815, exhibit a strong stimulatory capacity (Talmage *et al.*, 1977).

In the human system, similar claims have been made about the role of the equivalent HLA-DR determinants on stimulating cells (Albrechtsen & Lied, 1978, Gottlieb, Fu, Yu, Wang, Halper & Kunkel, 1979), and the ability of monoclonal heteroantisera

Correspondence: Dr L. K. Ashman, Department of Microbiology and Immunology, The University of Adelaide, G.P.O. Box 498, Adelaide 5001, South Australia, Australia.

0019-2805/82/1200-0633$02.00

against HLA-DR antigens to inhibit both allogeneic and autologous MLR suggests a major stimulatory role for HLA-DR molecules in both of these systems (Gottlieb *et al.*, 1979). However, as with the mouse system, the evidence is inconclusive, since HLA-DR positive cultured melanoma cells and chronic lymphocytic leukaemia B cells both fail to stimulate in MLR (Pollack, Livingston, Fogh, Carey, Oettgen & Dupoint, 1980; Halper, Fu, Gottlieb, Winchester & Kunkel, 1979). The reported function of the monocyte in MLR varies from an absolute requirement (Huber *et al.*, 1981) to a supportive function (Lemke & Opitz, 1976), or a lack of requirement at high cell densities (Geier & Cresswell, 1980). The clarification of these aspects of MLR, the role and nature of the stimulatory cell, and the requirements for accessory cells, is imperative for understanding the mechanisms of induction of a cellular immune response, and has important implications for manipulation of the immune response against allografts (Lafferty, 1980) and tumours (Oliver & Lee, 1979).

This study investigates the ability of cell lines derived from patients with leukaemia or lymphoma to stimulate allogeneic responder populations from healthy donors in MLR. The cell lines fall into three categories: (i) those which stimulate allogeneic responder T cells in the presence or absence of accessory (non-T) cells; (ii) those which stimulate T cells only in the presence of accessory cells; and (iii) those which fail to stimulate in either case. There was no correlation between the stimulatory capacity of these cell lines and the presence of serologically defined HLA-DR determinants.

MATERIALS AND METHODS

Preparation of responder cells

Mononuclear cells (MNC) from peripheral blood of healthy adult donors were prepared by buoyant density centrifugation using Sepalymph (Teva Pharmaceutical Industries Inc., Jerusalem), washed in RPM1 1640 supplemented with 10% heat-inactivated foetal calf serum (RPM1/10% FCS) and penicillin-streptomycin (100 u./ml) (Flow Laboratories) and resuspended at 2×10^6 cells per ml. Adherent cells were removed from the MNC by adherence to plastic at 37° for 1 hr and T cells were prepared according to the method of Madsen, Johnsen, Wendleboe Hanson & Christiansen, 1980). Briefly, equal volumes of 2×10^6 cells/ml non-adherent MNC and 0·5% aminoethyl-isothiuronium bromide treated sheep red blood cells (AET-SRBC) suspended in RPM1/10% FCS were incubated for 15 min at 37°, centrifuged at 50 *g* for 5 min and incubated at 4° for at least 1 hr. All FCS used in T-cell purification had been absorbed with equal volumes of packed SRBC for 1 hr at 4°. Rosette forming (E-RFC) and non-rosette forming cells (non-E-RFC) were separated by buoyant density centrifugation using Sephalymph and the AET-SRBC were lysed with 0·8% ammonium chloride in Tris buffer pH 7·6 at room temperature for 5 min. The E-RFC and non-E-RFC were washed in RPM1/10% FCS and resuspended in the same medium at appropriate concentrations.

Unfractionated MNC and E-RFC (T cells) were used as responder cells. Monocyte contamination of the T-cell preparation was less than 0·5% as assessed by non-specific esterase staining (Li, Lam & Yam, 1973), and B-cell contamination, determined by fluorescence analysis of surface immunoglobulin was less than 1%.

Cell lines

Balm-1, Cess B, U937, K562 and HL-60 were kindly provided by Dr David Jose, The Cancer Institute, Melbourne, RC2a was provided by Dr Ray Bradley, The Cancer Institute Melbourne, and HSB-2, JM and Molt-4 were kindly supplied by Dr H. Zola, Flinders Medical Centre, Adelaide. The HL-60 subline arose in our laboratory after treatment of HL-60 with amphotericin B, and has been maintained as a vigorously growing undifferentiated cell type for over 18 months. Unlike the parent HL-60 line, it cannot be induced to differentiate using DMSO (unpublished data).

Preparation of stimulator cells

Human leukaemic and lymphoblastoid cell lines were maintained in continuous suspension culture in RPM1/10% FCS. Cells to be used in MLR were harvested during the logarithmic phase of growth, washed in RPM1/10% FCS, and incubated for 1 hr at 37° with 20 μg of Mitomycin C (Sigma Chemical Company, St Louis, U.S.A.) per 2×10^6 cells in 1 ml of RPM1/10% FCS. The cells were washed in RPM1/10% FCS and resuspended to the appropriate concentration for the MLR.

Non-E-RFC, prepared from non-adherent MNC were treated for 1 hr at 37° with mitomycin C, as above, washed in RPM1/10% FCS and resuspended to the appropriate concentrations for the assay. This was a mixed population of cells and, depending on the

donor, contained greater than 50% B cells, varying numbers of null cells and monocytes and less than 5% T cells.

Preparation of accessory cells

Adherent cells were prepared by overnight incubation of MNC at 5×10^6 cells/ml in RPMI/10% FCS in humidified 7·5% CO_2, 7·5% O_2 in N_2. The non-adherent cells were removed by gentle washing and the adherent cells were removed by vigorous pipetting. The adherent cells were treated with mitomycin C as above, and were greater than 95% monocytes as determined by morphological criteria, non-specific esterase staining, and peanut agglutinin binding (O'Keefe & Ashman, 1982).

Non-E-RFC were depleted of any remaining adherent cells by repeated incubations in plastic petri dishes, until the level of monocytes was less than 1%. When tested for accessory cell function this population of cells contained B cells and Null cells, in varying proportions depending on the donor.

Preparation of supernatants

Culture supernatants from cell lines in logarithmic phase of growth were harvested and tested for the influence on MLR. In addition mitomycin C-treated K562 and Molt-4 cells were each incubated overnight with adherent cells at 37° in humidified 7·5% CO_2, 7·5% O_2 in N_2 and the supernatants collected for testing in MLR.

Mixed lymphocyte reaction

Triplicate cultures of 2×10^5 responder cells with 2×10^3 to 2×10^5 stimulator cells in 0·2 ml of RPMI/10% FCS were incubated at 37° in humidified 7·5% CO_2, 7·5% O_2 in N_2 for 6 days. The cultures were pulsed with 1 μCi per well of tritiated thymidine (Amersham) 18 hr before harvesting (Titertek cell harvester). The filter discs were placed in 2 ml ACS-II (Amersham) and counted using a Beckman LS 7500 scintillation counter. Variations to this procedure included addition of 1 μg/ml Indomethacin [Merck Sharp and Dohme (Australia) Pty Ltd] to the medium, addition of accessory cells, and addition of culture supernatants. The tritiated thymidine incorporation was calculated as the arithmetic mean of triplicate counts per minute $\pm$ the standard deviation. The standard deviation was less than 10% and is not included in some figures.

HLA-DR testing

All cell lines were tested for HLA-DR specificities by the Tissue Typing laboratory of the Red Cross Blood Transfusion Service in Adelaide. Cells were tested in the standard microcytotoxicity assay against a panel of alloantisera from multiparous females. The results confirmed those already reported for some cell lines (Boss, Delia, Robinson & Greaves, 1980; Gahmberg, Andersson & Nilsson, 1980; Minowada, Janossy, Greaves, Tsubota, Srivastava, Morikawa & Tatsumi, 1978; Koeffler, Billing, Sparkes, & Golde, 1980; Lozzio & Lozzio, 1979).

RESULTS

The cell lines investigated and some relevant characteristics are shown in Table 1. Cell lines were tested for mycoplasma contamination using three methods; direct culture, DNA staining by H stain, and inhibition of tritiated thymidine incorporation by phytohaemagglutinin-stimulated human lymphocytes (Dent, Cleland & Liao, 1980). All cell lines were mycoplasma free. The B-lymphoblastoid cell line Cess B, is autologous to the myelomonocytic cell line RC2a. All cell lines were tested as stimulator cells in MLR against purified T responders and MNC from at least two donors, and in some experiments three or four donors. In most cases, the MNC and T cells were not paired samples from the same donor. Each responder population was tested for the normal allogeneic response using non-E-RFC as stimulators, and in all experiments the stimulator cells alone incorporated less than 500 counts per minute (c.p.m.) of tritiated thymidine. Responder cell populations with unstimulated tritiated thymidine incorporation of greater than 20,000 c.p.m. were considered to be activated cells and were excluded from these results. T-cell and MNC responses to the cell lines are shown in Fig. 1. The cells were tested at responder:stimulator ratios from 1:1 to 200:1 against two or more responder populations. The numbers of responder cells were constant at 2×10^5 per well and the stimulator cell numbers varied from 2×10^5 per well to 2×10^3 per well in most cases, but to 1×10^3 per well with some cell lines. The cell lines fall into three groups as stimulator cells.

Group 1 cell lines stimulated allogeneic responder T cells and MNC. This group included RC2a, Cess B, Balm-1 and U937, which are HLA-DR positive and HSB2, an HLA-DR negative T-cell line. The maximum tritiated thymidine incorporation for Balm-1,

Table 1. Some relevant characteristics of cell lines investigated as stimulator cells in allogeneic MLR

Cell line	Origin	Main cell type	E-RFC	HTLA	HLA A,B,C	HLA-DR
MOLT-4	T-ALL	T cell	+	+	+	−
HSB-2	Childhood T-ALL	T cell	−	+	+	−
JM	T-ALL	T cell	+	+	+	−
HL-60	Promyelocytic leukaemia	Promyelocyte	−	−	+	−
HL-60 Subline	Spontaneous, After amphotericin B treatment	Blast cell	−	−	−	−
RC2a	AMML	Monocyte	−	−	+	+
Cess B	B-lymphoblastoid from AMML	B cell	−	−	+	+
BALM-1	B-ALL	B cell	−	−	+	+
U937	Histiocytic lymphoma	MO type	−	−	+	+
K562	CML-blast crisis	Myeloid blast	−	−	−(+)	−

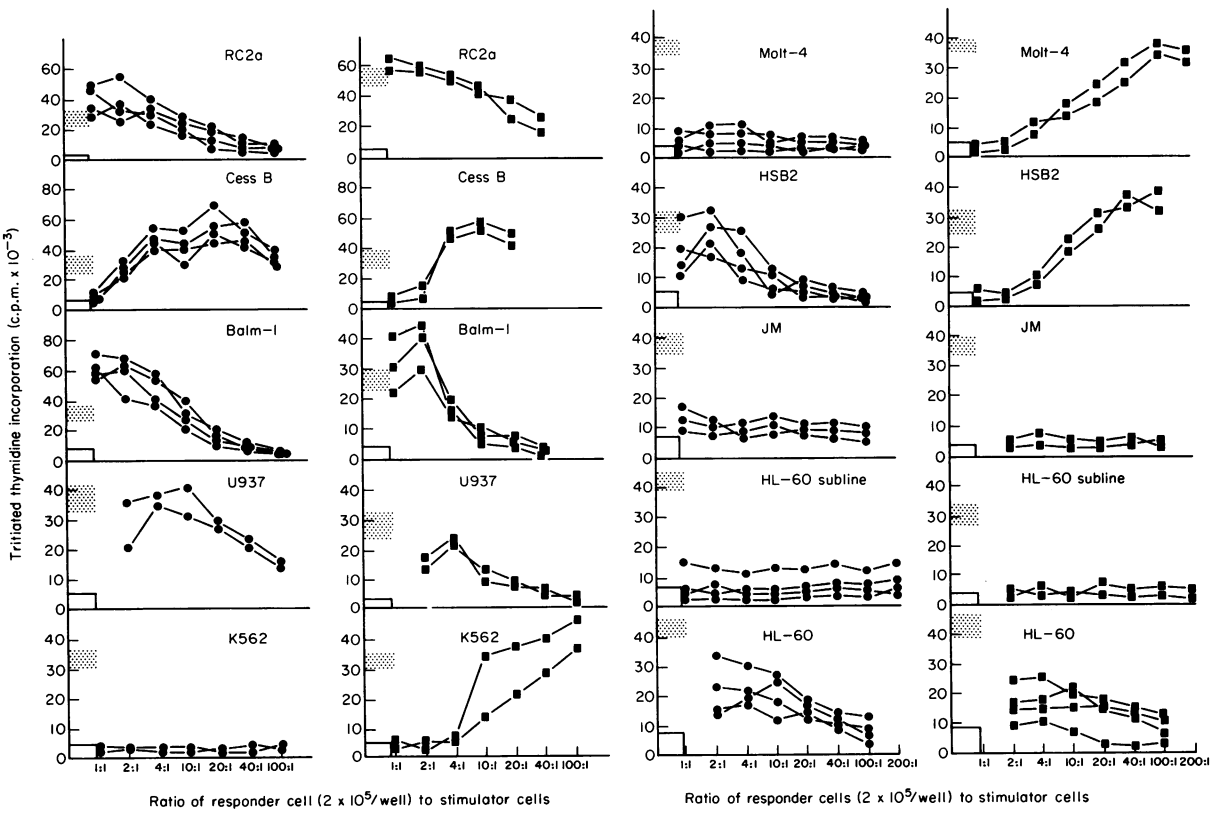

Figure 1. T-cell (●——●) and MNC (■——■) responses from two or more donors to human cell lines. The unstimulated tritiated thymidine incorporation ±2 SD (▭) and the response to allogeneic non-E-RFC (▨) for each group of responders is shown to the left of each graph.

RC2a and Cess B was often higher than that for the allogeneic non-E-RFC, while U937 and HSB2 exhibited a stimulating capacity similar to the non-E-RFC.

Group 2 cell lines, K562 and Molt-4, failed to stimulate T cells, but stimulated unfractionated MNC. Both cell lines are HLA-DR negative. The MNC response to K562 was restricted at high stimulator cell densities (from 2×10^5 to 5×10^4 cells per well) by metabolic requirements and a drop in pH, since addition of fresh medium to the culture on day 4, slightly enhanced the response at these cell densities. The response of MNC to Molt-4 was not changed by addition of fresh medium on day 4 (data not shown).

Group 3 cell lines, JM, HL-60 and HL-60 subline failed to stimulate MNC or T cells. While JM and HL-60 subline failed to stimulate at any responder: stimulator ratio tested, HL-60 exhibited a low stimulating capacity at ratios from 20:1 to 1:1, and was clearly different from the other cell lines in this group. It is included in this group, however, since it failed to stimulate T cells or MNC to the same level as the normal allogeneic non-E-RFC. Group 3 cell lines also failed to stimulate when assayed for varying times, up to 10 days (data not shown). Culture supernatants from logarithmic phase cell lines were found to have no effect on the unstimulated responder populations, or on the normal allogeneic response to non-E-RFC.

Figure 2 demonstrates the effect of adding increasing numbers of autologous mitomycin-C-treated adherent cells on the T-cell response to Molt-4. The adherent cells enhanced the T-cell response at low cell numbers, but as the numbers of adherent cells were increased, the response was reduced to a level below that of unstimulated T cells. Since prostaglandin E_2 production by monocytes has been reported to affect T-cell responses (Baker, Fahey & Munck, 1981) the MLR was repeated using 1 μg/ml indomethacin in the medium. Figure 3 demonstrates the effect of indomethacin on T-cell responses to K562 and Molt-4 in the presence and absence of adherent cells. The response of T cells in the presence of indomethacin and adherent cells was almost to the same level as the response of unfractionated MNC to K562 and Molt-4 (Fig. 3). These responses are dependent on cell number ratios, and the suboptimal responder: stimulator ratio of 4:1 was chosen for the experiments shown in Fig. 3,

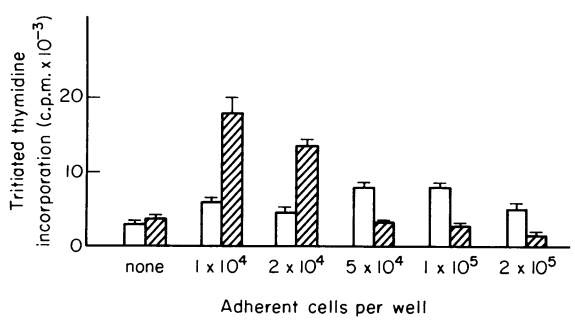

Figure 2. Effect of adding increasing numbers of autologous adherent cells to 1×10^5 unstimulated T cells (□), and to 1×10^5 T cells stimulated with 1×10^5 Molt-4 cells (■).

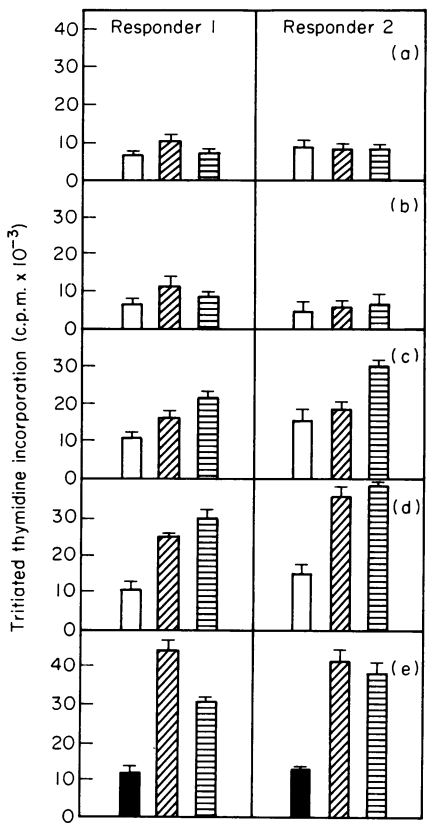

Figure 3. Effect of 1 μg/ml indomethacin on T-cell responses of two responders to K562 and Molt-4 in the presence and absence of autologous adherent cells. (a–d) (□) 1×10^5 T cells; (■) 1×10^5 T cells + $2{\cdot}5 \times 10^4$ K562; (▩) 1×10^5 T cells + $2{\cdot}5 \times 10^4$ Molt-4. (a) No indomethacin, no adherent cells; (b) +1 μg/ml indomethacin; (c) + $2{\cdot}5 \times 10^4$ adherent cells; (d) +1 μg/ml indomethacin + $2{\cdot}5 \times 10^4$ adherent cells; (e) response of MNC from the same two responders in the presence of 1 μg/ml indomethacin. (■) 1×10^5 MNC; (▩) 1×10^5 MNC + $2{\cdot}5 \times 10^4$ K562; (▩) 1×10^5MNC + $2{\cdot}5 \times 10^4$ Molt-4.

based on the expected response for unfractionated MNC at that ratio. Further experiments using different responder:stimulator ratios, in the presence and absence of adherent cells showed that enhanced responses to K562 could be obtained at higher responder:stimulator ratios (Fig. 4).

Addition of autologous non-adherent, non-E-RFC to T cells had a similar effect on the responses to K562 and Molt-4. Figure 5 demonstrates that equivalent numbers of autologous adherent cells, or autologous non-adherent, non-E-RFC, enhanced the T-cell responses of two different donors to K562 and Molt-4.

Supernatants from overnight culture of adherent cells with K562 or Molt-4 had no effect on the autologous T-cell responses to these cell lines. A similar result was obtained using supernatants of non-adherent, non-E-RFC incubated overnight with K562 or Molt-4.

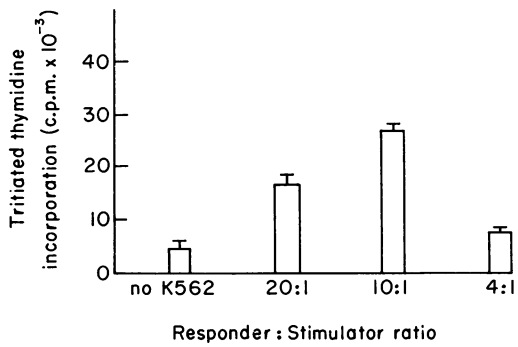

Figure 4. Effect of changing responder:stimulator cell ratios when 1×10^5 T cells are stimulated by K562 in the presence of $2{\cdot}5 \times 10^4$ autologous adherent cells and 1 μg/ml Indomethacin.

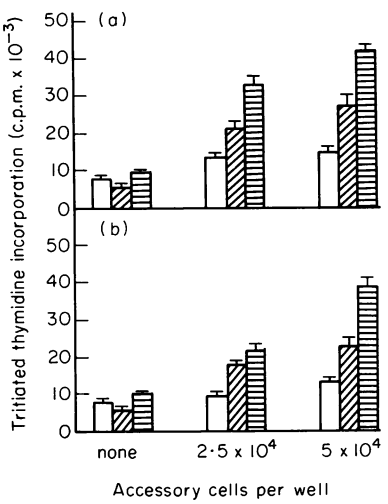

Figure 5. Effect of adding autologous adherent cells (a) or autologous non-adherent non-E-RFC (b) on the T-cell responses to K562 and Molt-4. Indomethacin was not used in this experiment. (□) 1×10^5 T cells; (■) 1×10^5 T cells + $2{\cdot}5 \times 10^4$ K562; (▩) 1×10^5 T cells + 5×10^4 Molt-4.

DISCUSSION

The classical MLR between allogeneic blood lymphocytes has been attributed to differences between responder and stimulator cells for the products of the HLA-DR locus. There is, however, increasing evidence that although the expression of the serologically defined DR determinants and the lymphocyte-defined D determinants (lymphocyte-activating determinants, LADs) is closely correlated, the two are not identical (Termijtelen & van Rood, 1981; van Heyningen, Cohen, Deane, Gray & Steel, 1981). The results of this study support such evidence.

Although most of the cell lines investigated in this study have been tested as stimulating cells in MLR by previous workers, the results are not uniform, and in many cases the cells were only tested at a few responder:stimulator ratios. Of particular interest is the stimulating capacity of the T-cell lines HSB2 and Molt-4. Both these lines have been investigated by many authors (Minowada *et al.*, 1978; Royston, Graze & Pitts, 1974; van Heyningen *et al.*, 1981) and found to be non-stimulatory for unfractionated MNC at responder:stimulatory ratios from 20:1 to 1:2. Similar results were obtained in this study at ratios from 1:1 to 4:1 when MNC were used as responders; however, a proliferative response was obvious at 10:1 and peaked at 100:1 for both HSB2 and Molt-4. The finding that these two HLA-DR negative cell lines stimulate in MLR supports the view that LADs are separate from the HLA-DR determinants.

The myeloid blast cell line K562 (Lozzio & Lozzio 1979) has been reported to be weakly stimulatory (van Heyningten *et al.*, 1981) and highly stimulatory (Han, Dadey & Minowada, 1977) for MNC responders. This disparity may be explained by the different techniques employed, and the variations in cell ratios. Our results demonstrate the importance of responder:stimulator ratios, since K562 is non-stimulatory at 4:1 and highly stimulatory at 100:1. In addition, K562 has high glucose and other metabolic requirements, and at high

cell densities (5×10^4–2×10^5 per well) quickly depletes the medium of nutrients, and lowers the pH. K562 is also a fairly large cell (from 7 to 55 μ diameter, and 30% of cells greater than 20 μ (Lozzio & Lozzio, 1975)) when compared with other cells investigated, and this may have some steric effect on lymphocyte activation. Despite a considerable degree of β_2-microglobulin synthesis and secretion, K562 does not express HLA antigens (Klein, Zeuthen, Eriksson, Terasaki, Bernoco, Rosen, Masucci, Povey & Ber, 1980) when tested using standard techniques. However, a recent report has shown that low levels of HLA heavy and light chains can be detected on the surface of K562 cells using monoclonal reagents (Ziegler, Laudien, Heinrichs, Müller, Uchanska-Ziegler & Wernet, 1981). K562 is also reported to be HLA-DR negative, by standard techniques and radioimmunoassay (Lozzio & Lozzio, 1979).

The disparity in stimulation by K562 and Molt-4 when different responder populations are used, raises several questions about the mechanisms involved in MLR. Despite the prevalence of reports describing the requirement for adherent cells or monocytes in MLR (Huber *et al.*, 1981; Lemke & Opitz, 1976) clearly a strong response can occur in the absence of significant numbers of monocytes (Gottlieb *et al.*, 1979; Geier & Cresswell, 1980). The purified T-cell responses to Balm-1, a B-cell line, in the absence of monocytes were similar to the responses of unfractionated MNCs. The responses to K562 and Molt-4 however, clearly show some requirement for an accessory cell. Our results indicate that either autologous adherent cells, or non-adherent, non-E-RFC can function as accessory cells in this response. Manipulation of the cells may be the reason why responses of T cells to Molt-4 and K562 in the presence of accessory cells were not as high as the unfractionated MNC response. The finding that non-adherent, non-E-RFC as well as adherent cells can function as accessory cells was surprising. This was not due to contamination of the non-adherent population with monocytes, but we cannot exclude the possibility that both adherent and non-adherent, non-E-RFC contain small numbers of dendritic cells which are responsible for the accessory function (Steinman & Nussenzweig, 1980). There are, however, no reports of dendritic cells in human peripheral blood, at this stage. [Note added in proof: human dendritic cells have now been purified from peripheral blood (Van Voorhis, Hair, Steinman & Kaplan, 1982).]

Activation of T cells by allogeneic cells in MLR is thought to be a two signal process, requiring antigen as signal 1 and a second non-antigen-specific signal which can be either Interleukin 1 or Interleukin 2 (IL-1, IL-2). Thus cells are classified as 'stimulators' or 'non-stimulators' in allogeneic MLR, depending on whether or not they can provide this second signal (for example, see Lafferty, Andrus & Prowse, 1980). In this context some cell lines appear to be capable of providing the second signal, for example, the myelomonocytic cell line RC2a, and the macrophage-like line, U937, which might be expected to be capable of producing IL-1. HSB2 is a T-cell line, and might produce IL-2 under some circumstances, although it does not do so constitutively, or in response to mitogens (Gootenberg *et al.*, 1981). The B-cell lines which stimulate in the absence of accessory cells, Balm-1 and Cess B, are not known to produce IL-1 or IL-2, however purified normal B cells have been shown to be stimulatory in allogeneic MLR (Gottlieb *et al.*, 1979). The presence of Epstein-Barr Virus may also have some influence on stimulatory capacity of Cess B.

In the case of the 'conditional stimulators', Molt-4 and K562, it is necessary to postulate that lymphokine is produced only in the presence of accessory cells. Figure 6 shows three possible mechanisms by which this might occur.

(a) Soluble antigen from K562 or Molt-4 is being presented on the accessory cell to the T cells, i.e. indirect presentation. Although monocytes have been described as accessory cells for antigen presentation (Huber *et al.*, 1981) non-adherent, non-E-RFC have not been reported to act as accessory cells in the human system. However a recent report suggests B tumour cells may be capable of antigen presentation in the murine system (McKean, Infante, Nilson, Kimoto, Fathman, Walker & Warner, 1981).

(b) The autologous MLR of T cells responding to accessory cells (Weksler, & Kozak, 1977) is providing the second signal for the response to K562 and Molt-4.

(c) The third possible explanation is that K562 and Molt-4 carry the necessary receptor (the nature of which is at present unknown) to trigger IL-1 production by allogeneic accessory cells, and this can act as signal 2 for the T cell/Molt-4 or T cell/K562 interaction.

Further studies are in progress in order to determine whether any of these models are applicable. Of obvious importance, but not included in this study is the cell type of responder T cells. Cytotoxicity studies using various cell lines for priming and as targets, and use of OKT sera for cell typing (Reinherz, Kung,

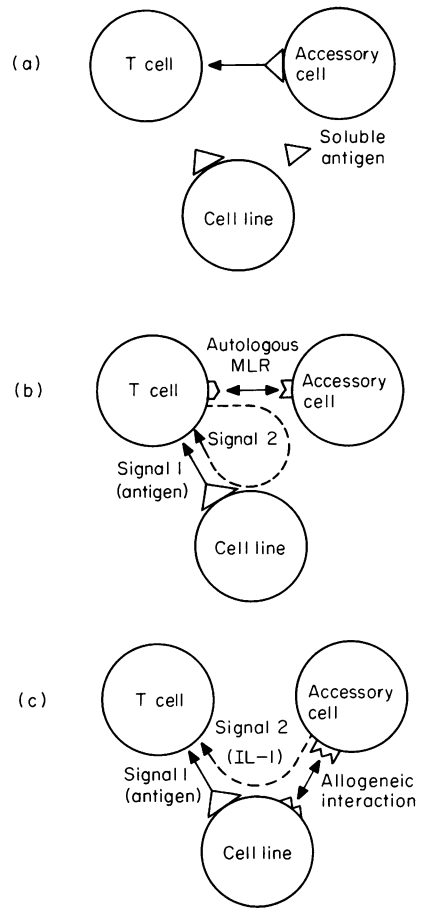

Figure 6. Possible mechanisms of action involved in the T-cell response to K562 and/or Molt-4 in the presence of accessory cells. (a) Indirect presentation of antigen; (b) signal 2 produced by T cell/accessory cell autologous MLR; (c) signal 2 produced by cell line/accessory cell allogeneic interaction.

Goldstein & Schlossman, 1979) would be the next step in clarifying this area.

It seems therefore that human leukaemic cell lines display a wide range in stimulatory capacity, and that no clear cut definition of 'stimulator' or 'non-stimulator' can be applied to cells without some study of mechanisms involved in lymphocyte activation. The widely held assumption that T-cell lines do not stimulate in MLR has been the result of failure to critically evaluate original studies, and acceptance of the concept that HLA-DR antigens and LADs are identical. Similarly the view that accessory cells are an absolute requirement for lymphocyte activation fails to account for the many reports of activation without accessory cells. The results of this study may shed light on the lack of stimulatory capacity of patient leukaemic cells, and have clinical application in the areas of immune response manipulation and enhancement in leukaemia and tumour patients.

ACKNOWLEDGMENTS

This work was supported by grants from the National Health and Medical Research Council of Australia and the Anti-Cancer Foundation of the Universities of South Australia. D. E. O'Keefe is an Anti-Cancer Foundation Research Associate; Dr L. K. Ashman is a Rotary Peter Nelson Leukaemia Research Fellow. We are grateful to the Australian Red Cross Society for the normal blood samples, and to Ms Jill Furze for technical assistance.

REFERENCES

ALBRECHTSON D. & LIED M. (1978) Stimulating capacity of human lymphoid cell subpopulations in mixed lymphocyte cultures. *Scand. J. Immunol.* **7,** 427.

BAKER P.E., FAHEY J.V. & MUNCK A. (1981) Prostaglandin inhibition of T-cell proliferation is mediated at two levels. *Cell. Immunol.* **61,** 52.

BOSS M.A., DELIA D., ROBINSON J.B. & GREAVES M.F. (1980) Differentiation-linked expression of cell surface markers on HL-60 leukaemic cells. *Blood,* **56,** 910.

DAUSSET J. & FRADELIZI D. (1977) Histocompatibility antigens, mixed lymphocyte reaction genes and transplantation. In: *B and T cells in Immune Recognition* (Ed. by F. Loor and G.E. Roelants), p. 407, Wiley, New York.

DENT P.B., CLELAND G.B. & LIAO S.-K. (1980) Detection and control of occult mycoplasma contamination in human tumour cell lines. *Cancer Immunol. Immunother.* **8,** 27.

GAHMBERG C.G., ANDERSSON L.C. & NILSSON K. (1980) Surface glycoproteins of human non-T, non-B acute lymphocytic leukaemia cell lines. *Leukaemia Res.* **4,** 279.

GEIER S.S. & CRESSWELL P. (1980) Monocytes as accessory cells in human mixed lymphocyte response: lack of requirement at high cell densities. *Cell Immunol.* **49,** 190.

GOOTENBERG J.E., RUSCETTI F.W., MIER J.W., GASDAR A. & GALLO R.C. (1981) Human cutaneous T cell lymphoma and leukaemia cell lines produce and respond to T cell growth factor. *J. exp. Med.* **154,** 1403.

GOTTLIEB A.B., FU S.M., YU D.T.Y., WANG C.Y., HALPER J.P. & KUNKEL H.G. (1979) The nature of the stimulatory cell in human allogeneic and autologous MLC reactions; role of isolated IgM-bearing B cells. *J. Immunol.* **123,** 1497.

GREINEDER D.K., SHEVACH E.M. & ROSENTHAL A.S. (1976) Macrophage-lymphocyte interaction. III. Site of alloantiserum inhibition of T lymphocyte proliferation induced by allogeneic or aldehyde-bearing cells. *J. Immunol.* **117,** 1261.

HALPER J.P., FU S.M., GOTTLIEB A.B., WINCHESTER R.J. & KUNKEL H.G. (1979) Poor mixed lymphocyte reaction stimulatory capacity of leukaemic B cells of chronic lymphocytic leukaemia patients despite the presence of Ia antigens. *J. clin. Invest.* **64,** 1141.

HAN T., DADEY B. & MINOWADA J. (1979) Cultured human leukaemic non-T, non-B lymphoblasts and their stimulating capacity in 'one-way' mixed lymphocyte reaction. Suggestive evidence for early T-cell or B-cell precursors. *Cancer*, **44,** 136.

HUBER, C., FINK U., LEIBOLD W., SCHMALZL F., PETERSON P.A., KLARESKOG L. & BRAUNSTEINER H. (1981) The role of adherent HLA-DR$^+$ mononuclear cells in autologous and allogeneic MLR. *J. Immunol.* **127,** 726.

KLEIN G., ZEUTHEN J., ERIKSSON I., TERASAKI P., BERNOCO M., ROSEN A., MASUCCI G., POVEY S. & BER R. (1980) Hybridization of a myeloid leukaemia-derived human cell line (K562) with a human Burkitt's lymphoma line (P3HR-1). *J. natn. Cancer Inst.* **64,** 725.

KOEFFLER H.P., BILLING R., SPARKES R.S. & GOLDE D.W. (1980) Antigens present on human myeloid leukaemia cell lines. *Leukaemia Res.* **4,** 69.

LAFFERTY K.J. (1980) Immunogenicity of foreign tissues. *Transplantation*, **29,** 179.

LAFFERTY K.J., ANDRUS L. & PROWSE S.J. (1980) Role of lymphokine and antigen in the control of specific T cell responses. *Immunol. Rev.* **51,** 279.

LEMKE H. & OPITZ H.G. (1976) Function of 2 mercaptoethanol as a macrophage substitute in the primary immune response *in vitro*. *J. Immunol.* **117,** 388.

LI C.Y., LAM K.W. & YAM L.T. (1973) Esterases in human leukocytes. *J. Histochem. Cytochem.* **18,** 901.

LOZZIO C.B. & LOZZIO B.B. (1975) Human chronic myelogenous leukaemia cell-line with positive Philadelphia Chromosome. *Blood*, **45,** 321.

LOZZIO B.B. & LOZZIO C.B. (1979) Properties and usefulness of the original K-562 human myelogenous leukaemia cell line. *Leukaemia Res.* **3,** 363.

MCKEAN D.J., INFANTE A.J., NILSON A., KIMOTO M., FATHMAN C.G., WALKER E. & WARNER N. (1981) Major histocompatibility complex-restricted antigen presentation to antigen-reactive T cells by B lymphocyte tumor cells. *J. exp. Med.* **154,** 1419.

MADSEN M., JOHNSEN H.E., WENDLEBOE HANSEN P. & CHRISTIANSEN S.E. (1980) Isolation of human T and B lymphocytes by E-Rosette gradient centrifugation. Characterization of the isolated subpopulations. *J. immunol. Meths*, **33,** 323.

MEO T., DAVID C.S. & SHREFFLER D.C. (1976) H-2 associated MLR determinants: immunogenetics of the loci and their products. In: *The Role of Products of the Histocompatibility Gene Complex in Immune Responses*, p. 167. Academic Press, Inc., New York.

MINOWADA J., JANOSSY G., GREAVES M.F., TSUBOTA T., SRIVASTAVA B.I.S., MORIKAWA S. & TATSUMI E. (1978) Expression of an antigen associated with acute lymphoblastic leukaemic in human leukaemia-lymphoma cell lines. *J. natn. Cancer Inst.* **60,** 1269.

NIEDERHUBER J. (1978) Role of I region gene products in macrophage-T lymphocyte interactions. *Immunol. Rev.* **40,** 28.

O'KEEFE D.E. & ASHMAN L.K. (1982) Peanut agglutinin. A marker for normal and leukaemic cells of the monocyte lineage. *Clin. exp. Immunol.* **48,** 329.

OLIVER R.T.D. & LEE S.K. (1979) Histocompatibility antigens and T-cell responses. *Haematol. Blood Trans.* **23,** 377.

POLLACK M.S., LIVINGSTONE P.O., FOGH J., CAREY T.E., OETTGEN H.F. & DUPONT B. (1980) Genetically appropriate expression of HLA and DR(IA) alloantigens on human melanoma cell lines. *Tissue Antigens*, **15,** 249.

REINHERZ E.L., KUNG P.C., GOLDSTEIN G. & SCHLOSSMAN S.F. (1979) Separation of functional subsets of human T cells by monoclonal antibody. *Proc. natn. Acad. Sci. (USA)*, **76,** 4061.

ROYSTON I., GRAZE P.R., & PITTS R.B. (1974) Failure of cultured human T-cell lymphoid lines to stimulate a mixed leukocyte culture. *J. natn. Canc. Inst.* **33,** 361.

STEINMAN R.M. & NUSSENZWEIG M.C. (1980) Dendritic cells: features and functions. *Immunol. Rev.* **53,** 127.

TALMAGE D.W., WOOLNOUGH J.A., HEMMINGSEN H., LOPEZ L. & LAFFERTY K.J. (1977) Activation of cytotoxic T cells by non-stimulating tumour cells and spleen cell factor(s). *Proc. natn. Acad. Sci (USA)*, **74,** 4610.

TERMIJTELEN A. & VAN ROOD J.J. (1981) The role in primary MLC of the non HLA-D/DR determinant PL3A. *Tissue Antigens*, **17,** 57.

VAN HEYNINGEN V., COHEN B.B., DEANE D.L., GRAY C. & STEEL C.M. (1981) Serological distinction between DR antigens and lymphocyte activating determinants. *Tissue Antigens*, **17,** 149.

VAN VOORHIS W.C., HAIR L.S., STEINMAN R.M. & KAPLAN G. (1982) Human dendritic cells. Enrichment and characterization from peripheral blood. *J. exp. Med.* **155,** 1172.

WEKSLER E.M. & KOZAK R. (1977) Lymphocyte transformation induced by autologous cells. V. Generation of immunologic memory and specificity during the autologous mixed lymphocyte reaction. *J. exp. Med.* **146,** 1833.

WOLOS J.A. & DAVEY F.R. (1980) Function of lymphocyte subpopulations in chronic lymphocytic leukaemia. Activity in the allogeneic and autologus mixed lymphocyte reaction. *Cancer*, **45,** 893.

ZIEGLER A., LAUDIEN D., HEINRICHS H., MÜLLER C., UCHANSKA-ZIEGLER B. & WERNER P. (1981) K562 cells express human Major Histocompatibility Antigens. *Immunogenetics*, **13,** 359.