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Presentation of a soluble bacterial antigen and cell-surface alloantigens by large granular lymphocytes (LGL) in comparison with monocytes

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SUMMARY

The ability of large granular lymphocytes (LGL) to function as antigen-presenting cells (APC) in the proliferative response to the soluble bacterial antigen streptolysin O (SLO) was investigated. Despite the fact that a subset of LGL isolated by sorting peripheral blood lymphocytes with the B73.1 monoclonal antibody on a fluorescence-activated cell sorter (FACS-IV) expressed MHC Class II molecules of the DP, DQ and DR subregion loci, presentation of SLO by LGL was not demonstrated. Thus, T-cell populations containing LGL but carefully depleted of monocytes, isolated either by sorting using the FACS-IV or by SRBC-rosetting, were unresponsive to antigenic stimulation with SLO. Application of exogenous interleukin-1 to FACS-IV-isolated LGL-containing T-cell populations did not elicit presentation of SLO by the LGL. *In vitro* activation with phytohaemagglutinin and interleukin-2, which induced Class II expression in T-cell populations, resulted in an increased expression of Class II molecules of the DP, DQ and DR specificities on LGL. Although such activated T-cell and LGL populations were incapable of presenting SLO to freshly isolated antigen-non-responsive T cells, both activated populations were able to act as stimulators in an allogeneic mixed lymphocyte reaction. The ability of highly Class II-positive activated LGL to present membrane-bound antigens suggests that their inability to present a soluble antigen may be related to the absence of effective antigen sequestration and/or processing mechanisms.

INTRODUCTION

An essential step in the initiation of either humoral or cell-mediated immunity to all thymus-dependent antigens is the activation of antigen-specific helper T cells (Th). Recognition of antigen by Th is dependent upon presentation of the antigen, or a processed form of the antigen, in association with MHC Class II molecules on the surface of antigen-presenting cells (APC). A second signal in the activation of Th is provided by APC through production of the immunoregulatory molecule interleukin-1 (IL-1), which induces the release of interleukin-2 (IL-2) by and/or the expression of IL-2 receptors on Th. The principal APC among peripheral blood mononuclear cells (PBMC) has generally been recognized to be the monocyte (Mo) (Unanue, 1984) or a subset of Mo (Raff, Picker & Stobo, 1980; Gonwa *et al.*, 1983; Zembala *et al.*, 1984). However, recent evidence has suggested that the APC function of Mo populations may be mainly due to contaminating dendritic cells (DC) (van Voorhis *et al.*, 1983; Bjercke & Gaudernack, 1985), and that peripheral blood large granular lymphocytes (LGL) can also exert APC function (Scala *et al.*, 1985).

LGL comprise a non-adherent, non-phagocytic population of lymphoid cells characterized by a reniform nucleus and cytoplasmic azurophilic granules and possessing a receptor for the Fc region of IgG (FcR) (Timonen *et al.*, 1979a, b; Timonen, Ortaldo & Herberman, 1981). This morphologically homogenous population is, however, phenotypically and functionally heterogeneous. In the past, interest in LGL has primarily focussed on the cytotoxic subset, operationally known as natural killer (NK) cells, by virtue of their ability to lyse certain tumour and virus-infected targets spontaneously and without prior sensitization (see Herberman, 1982). However, it is equally apparent that they may also be involved in interactions with normal cells. For example, the ability of LGL to lyse both allogeneic and autologous bone marrow cells (Hansson, Kiessling & Andersson, 1981) suggests that they may be involved in bone marrow transplant rejection and in the regulation of haematopoiesis. LGL may also play a role in the regulation of B-cell activity (Arai *et al.*, 1983; Tilden, Abo & Balch, 1983; Abruzzo & Rowley, 1983; Brieva, Targan & Stevens, 1984; Pistoia *et al.*, 1985) and may influence multifarious aspects of host immune defence mechanisms through the production of cytokines such as interferons, IL-1 and IL-2 (Kasahara *et al.*, 1983; Scala *et al.*, 1984).

The ability of a subset of LGL to produce IL-1 combined with the expression of MHC Class II molecules by the same subset of LGL (Scala *et al.*, 1984) implies that they may be effective APC. Indeed, evidence has recently been produced

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purporting to demonstrate that LGL are as effective in the presentation of soluble antigens to Th as Mo (Scala *et al.*, 1985). We have here investigated the ability of LGL to present the soluble antigen streptolysin O (SLO) to T cells but have found no evidence to support the claim that LGL may function as APC in this respect. However, activated LGL, upon which Class II expression is markedly increased, were effective stimulators in allogeneic mixed lymphocyte reactions (MLR), in common with activated T cells.

MATERIALS AND METHODS

Media

RPMI-1640 (Flow Laboratories Ltd, Irvine, Ayrshire) culture medium buffered with 0·2% sodium bicarbonate and supplemented with 200 μg/ml ampicillin, 200 μg/ml streptomycin and 2 mM L-glutamine was used throughout. In addition, media were supplemented with either 10% heat-inactivated newborn calf serum (RPMI-NCS) (Gibco Ltd, Paisley, Renfrewshire) or with 10% irradiated (40 Gy) autologous plasma (RPMI-AP).

Isolation of cell populations

PBMC were obtained by fractionation of heparinized peripheral blood samples from healthy volunteers on lymphocyte separation medium (Flow) and were washed twice with phosphate-buffered saline and resuspended in RPMI-NCS. T-cell populations were isolated from PBMC by the sequential application of three purification procedures. (i) PBMC ($2–5 \times 10^6$ cells/ml in RPMI-NCS) were incubated in horizontally positioned plastic tissue culture flasks (Nunc, Paisley, Renfrewshire) for 1–2 hr at 37° in 5% CO_2 in air. Non-adherent cells were removed by washing twice with RPMI-NCS and once with RPMI-AP. When culture flasks from which the non-adherent cells had been removed were incubated for a further 18 hr in RPMI-AP, a large proportion of the previously plastic-adherent cells became non-adherent. Such transiently adherent populations contained >90% Mo, as assessed by the criterion of non-specific esterase (NSE) positivity (Yam, Li & Crosby, 1971). (ii) B cells and residual Mo were depleted from 1–2 hr plastic-non-adherent populations by passage through nylon-wool columns (Julius, Simpson & Herzenberg, 1973). (iii) Final purification of T cells from nylon-wool eluted cells was carried out either by SRBC-rosetting or by sorting with a fluorescence-activated cell sorter (FACS IV, Becton Dickinson, Sunnyvale, CA). The rosetting procedure employed SRBC that had been pretreated with neuraminidase (Weiner, Bianco and Nussenzweig, 1973). In some cases, two cycles of SRBC-rosetting were required to separate T-cell populations from all detectable Mo. The FACS IV was used to fractionate nylon-wool-non-adherent cells according to their reactivity with the monoclonal antibody (MoAb) B73.1, a MoAb that recognizes an epitope of the FcR on LGL (Perussia *et al.*, 1983). Cells were labelled by indirect immunofluorescence and sorted into $B73.1^+$ (LGL) and $B73.1^-$ (T-cell) populations. Concomitant with the FACS IV sorting procedure, there was a reduction in the level of monocytes in the sorted cell populations, generally to an undetectable level. This monocyte depletion may have occurred by exclusion of monocytes from the sorting gates through the exclusion of large cells.

Antigen-presentation assay

Assays were conducted in triplicate in round-bottomed microwells of 96-well culture plates (Nunclon, Nunc, or Titertek, Flow Laboratories Ltd) in RPMI-AP supplemented with $2·5 \times 10^{-5}$ M 2-mercaptoethanol (BDH Chemicals Ltd, Poole, Dorset). Responding cell populations were added to microwells at a density of $2·5 \times 10^5$ cells/well and in some experiments irradiated (40 Gy) monocytes, LGL or activated T cells were also added to the wells. Responding populations were challenged with the soluble bacterial antigen SLO (Wellcome Ltd, Beckenham, Kent) at a final concentration of 0·1 IU/ml. For determination of the ability of cell populations to stimulate in an MLR, responding T-cell populations in RPMI-AP were cultured in microwells at a density of 1×10^5 cells/well with varying numbers of irradiated (40 Gy) stimulator cells. The stimulator cells used were either freshly isolated or had been activated by a 7-day *in vitro* culture in RPMI-AP supplemented with 1·0 μg/ml phytohaemagglutinin (PHA; Wellcome Ltd) and 20% concentrated IL-2 (prepared from the MLA-144 cell line as described by Roberts & Moore, 1985). The final total volume in all assay wells was adjusted to 200 μl and cultures were incubated for 6 days at 37° in 5% CO_2 in air. Cell proliferation was determined by pulsing with 1 μCi/microwell of tritiated thymidine ([^{3}H]TdR sp.act. 25 Ci/mol, Amersham International, Amersham, Bucks) for the last 16 hr of the culture period. Cells were harvested with an automated multi-well harvester (Dynatec Laboratories Ltd, Billingshurst, Sussex) onto glass fibre filters, and [^{3}H]TdR incorporation was determined by liquid scintillation counting.

Cytotoxicity assay

The K562 cell line was used as a target for the determination of LGL-mediated natural cytotoxicity. Target cells were labelled with 200 μCi sodium (^{51}Cr) chromate (Amersham International), washed several times and added to Luckham LP3 tubes (1×10^4 cells in 200 μl). A further 200 μl of effector cells were added to give effector:target ratios (E:T) between 20:1 and 80:1. Maximum isotope release was determined by adding Triton X-100 (1:50 dilution; Sigma Chemical Co, Poole, Dorset) to target cells and spontaneous release was assessed with tubes containing targets alone. After an 18 hr incubation at 37°, 5% CO_2, 200 μl of supernatant were harvested from each tube and both the cell pellet and the corresponding supernatant sample were counted in a gamma counter. Percentage cytotoxicity was calculated according to the formula:

$$\% \text{ cytotoxicity} = \frac{\%\ ^{51}\text{Cr test release} - \%\ ^{51}\text{Cr spontaneous release}}{\%\ ^{51}\text{Cr maximum release} - \%\ ^{51}\text{Cr spontaneous release}} \times 100.$$

MHC Class II expression

Cytocentrifuge cell preparations were stained with MoAb reactive with MHC Class II molecules (as listed in Table 1) by an immunoalkaline staining technique (Cordell *et al.*, 1984). In brief, acetone-fixed cell preparations were incubated, in sequence, with the primary mouse MoAb, with rabbit anti-mouse Ig (Dakopatts, Copenhagen, Denmark) and with an alkaline phosphatase anti-alkaline phosphatase complex (Dakopatts). The alkaline phosphatase reaction product was visualized with naphthol AS-MX phosphate (Sigma Chemical Co.) and Fast Red TR salt (Sigma Chemical Co.) as substrate.

Table 1. Monoclonal antibodies reactive with MHC Class II molecules

Monoclonal antibody	Specificity*	Reference/source
CR3/43	DR/DP/DQβ	Sunderland *et al.* (1981)
DA6.231	DR/DP > DQβ	Guy *et al.* (1982)
DA6.147	DR > DPα	Guy *et al.* (1982)
DA6.164	DRβ	van Heyningen *et al.* (1982)
Tu22	DQ	Ziegler *et al.* (1981)
MHM4	DP (?α/β)	Makgoba, Hildreth & McMichael (1983)

* Specificity assigned at Edinburgh Workshop (Steel, 1984).

RESULTS

Monocyte dependence of T-cell proliferative response to SLO

The level of SLO-induced T-cell proliferation was apparently dependent upon the level of monocytes in the responding T-cell population, as assessed by NSE staining (Fig. 1). Initial depletion of Mo (to < 1% NSE positive cells) from PBMC by adherence to plastic and passage through a nylon wool column consistently resulted in an increased level of proliferation in response to SLO. Further Mo depletion by one or, if necessary, two cycles of SRBC-rosetting (rosetting cells) or by FACS sorting (B73.1$^-$ cells) gave populations that were consistently antigen non-responsive. Both the rosetting and B73.1$^-$ cell populations could be rendered antigen-responsive by the addition of purified populations of Mo.

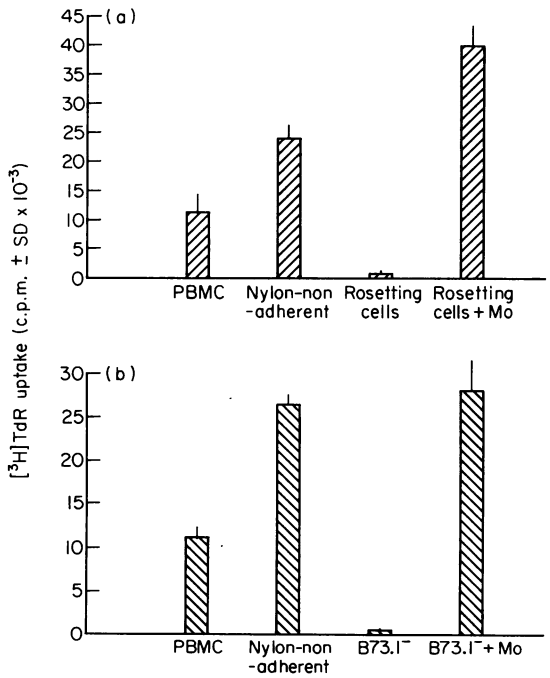

Figure 1. Monocyte dependence of T-cell response to SLO. Cells were cultured ($2{\cdot}5 \times 10^5$/well) in the presence of SLO (0·1 U/ml). Antigen-non-responsive T-cell populations were isolated by either (a) SRBC-rosetting, or (b) FACS-sorting with the B73.1 monoclonal antibody. The levels of monocytes in responding cell populations as determined by NSE staining were as follows: (a) PBMC 28%; nylon-non-adherent 0·2%; rosetting cells < 0·05%; (b) PBMC 34%; nylon-non-adherent 0·5%; B73·1$^-$ < 0·05%. Irradiated monocytes (40 Gy) were added back at $2{\cdot}5 \times 10^4$/well.

Expression of MHC Class II molecules by freshly isolated and *in vitro*-activated LGL

Since Class II positivity is a pre-requisite for APC function, it was important to confirm the status of LGL with respect to these products. Cytocentrifuge preparations of FACS-isolated LGL were stained with MoAb reactive with Class II framework determinants and with MoAb reactive with the DP, DQ and DR sublocus specificities by the APAAP technique. Staining with the framework MoAb showed that a subpopulation of LGL did express Class II antigens. The LGL stained in this way had been incubated at 37° (for 18 hr) prior to cytospin preparation to allow capping of bound B73.1 MoAb. Control slides in which the primary MoAb was replaced with buffer showed no positive staining, indicating that positivity was due to the presence of Class II antigens on LGL, rather than residual bound B73.1. Table 2 shows that the percentage Class II positivity of LGL varied widely from donor to donor (7–87%). The populations were also stained with MoAb reactive with the DP, DQ and DR sublocus specificities, and this showed that although all three types of Class II products were detectable on some LGL, the subpopulations of LGL expressing DP and DQ molecules were smaller than the DR-positive subpopulation (Table 2). A similar distribution of Class II subtypes was also seen upon staining of Mo populations. Table 2 also shows the effect of *in vitro* activation with PHA and IL-2 on Class II expression by LGL and T cells. The inducible expression of Class II antigens on T cells activated in culture by a variety of agents is well documented (Ko *et al.*, 1979; Reinherz *et al.*, 1979). Our results confirm this and show, additionally, that *in vitro* activation of LGL results in an increase in their Class II positivity. In fact, in our hands, activated LGL consistently expressed higher levels of Class II antigens than activated T cells. This inducibility of Class II antigens may indicate that expression of these products in freshly isolated LGL is a reflection of their activation state.

Failure of FACS-isolated LGL to support SLO-induced T-cell proliferation

Evidence that FACS-sorted LGL would not support the SLO-induced proliferative response came from experiments in which nylon-non-adherent cell populations were passed through the FACS as a final Mo depletion step. No fluorescent label was attached to the cells, but the population was subject to a purification imposed by the gating procedure, with both large and small cells being excluded from the sorted population. Such FACS-sorted populations (containing both B73.1$^+$ and B73.1$^-$ cells) were unresponsive to stimulation with SLO (Fig. 2). Responsiveness could be restored by the addition of purified populations of Mo.

Predictably, in experiments in which pure FACS-isolated T-cell populations (B73.1$^-$ cells) were reconstituted with FACS-isolated LGL (B73.1$^+$ cells) containing no detectable Mo, the proliferative response to SLO was not restored. However,

Table 2. Expression of MHC Class II molecules by LGL (B73.1+), T cells (B73.1−) and monocytes (Mo)

Donor	Cell type	Monoclonal antibody					
		CR3/43	DA6.231	DA6.147	DA6.164	Tu22	MHM4
CB	B73.1+ (freshly isolated)	36·1	31·8	15·1	25·2	17·0	16·3
	B73.1+ (activated)	53·3	55·1	23·7	36·3	29·2	ND
	B73·1− (freshly isolated)	<0·05	0·2	<0·05	<0·05	<0·05	ND
	B73.1− (activated)	ND	13·6	1·9	3·5	3·0	ND
	Mo	83·4	87·1	78·2	85·5	27·1	8·3
DH	B73.1+ (freshly isolated)	7·2	11·7	3·6	6·4	5·7	1·8
	B73.1+ (activated)	93·2	80·4	89·5	82·0	ND	79·0
	B73·1− (freshly isolated)	0·4	2·6	0·05	0·1	<0·05	ND
	B73·1− (activated	37·4	37·2	13·3	27·3	8·4	ND
MM	B73.1+ (freshly isolated)	87·1	83·7	6·8	12·9	3·7	4·3
	B73.1+ (activated)	77·2	82·0	74·7	74·1	67·6	ND
	B73·1− (freshly isolated)	10·2	ND	0·05	0·1	<0·05	ND
	B73·1− (activated)	50·3	24·2	14·8	22·4	7·6	ND

Values are the percentage cells positive by immunocytochemical staining (see text). Controls in which the primary MoAb was replaced with buffer were negative. B73.1+ and B73.1− cells were activated by a 7-day culture in round-bottomed microwells (1×10^5/well) with PHA (1·0 μg/ml) and IL-2 (20%).

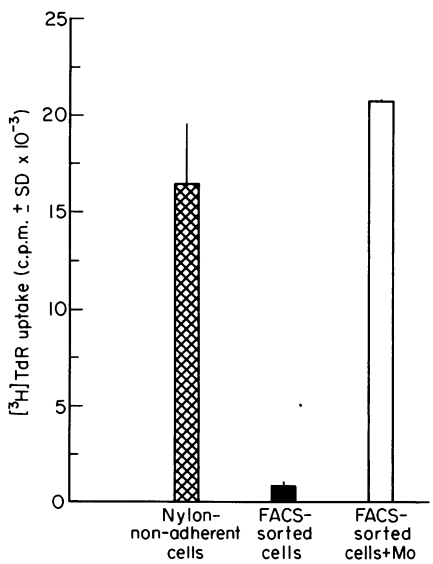

Figure 2. Effect of FACS-sorting on SLO-induced proliferative response. Cells were cultured ($2·5 \times 10^5$/well) with SLO (0·1 U/ml). FACS-sorted cells had been purified by the FACS-gating process and contained both T cells and LGL. Irradiated monocytes (40 Gy) were added back to FACS-sorted populations at $2·5 \times 10^4$/well.

B73.1+ populations containing a Mo contamination level of 0·3%, for example, did restore the SLO-induced response (data not shown). Such results illustrate the fact that very low levels of Mo (estimated to be as low as 0·05%) can be effective in antigen presentation, and thus that such contamination could lead to an incorrect interpretation of results with regard to the APC activity of contaminated cell populations.

Presentation of soluble and membrane-bound antigen by activated cells

Following the observation that LGL Class II expression was increased by *in vitro* activation, the ability of such populations to present soluble antigens to fresh autologous T cells was investigated. Numerous experiments indicated unequivocally that highly Class II-positive activated LGL were inefficient at, or incapable of, presenting SLO to antigen-non-responsive T-cell populations (data not shown). Activated T cells containing a Class II-positive subpopulation were also unable to present SLO to freshly isolated autologous T cells (data not shown).

The inability of activated Class II-positive cells to present soluble antigens such as SLO could be due to the absence in such cells of a suitable antigen-processing mechanism. Since activation of Th by allogeneic MHC products is independent of a processing step (Lee, Wong & Spitzer, 1982), it was of interest to investigate the ability of activated T cells and LGL to stimulate an allogeneic MLR (Fig. 3).

As anticipated, an MLR was generated by stimulation of pure T-cell populations with allogeneic PBMC. The stimulatory component of such PBMC was identified as the monocyte population, since pure Mo, but not pure T cells, were effective in the generation of an MLR in allogeneic responder cells. Fresh LGL were incapable of generating an allogeneic MLR at the responder: stimulator ratios used, although the ratios that could be tested were restricted by the low numbers of LGL available by FACS sorting.

By contrast, both T cells and LGL were able to stimulate an allogeneic MLR after activation with PHA and IL-2. Such stimulation was not the result of alloantigen presentation by residual autologous accessory cells in the responding T-cell populations, since an MLR was not generated if responder T cells were challenged with allogeneic T cells not expressing Class II antigens, i.e. fresh allogeneic T cells or allogeneic T cells

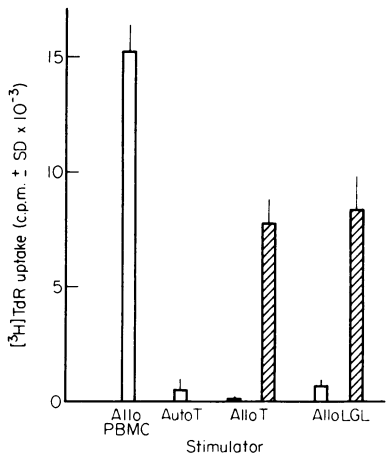

Figure 3. Stimulation of an allogeneic MLR by activated LGL and activated T cells. Responder T cells (1×10^5/well) were cultured with irradiated stimulator cells (2×10^4/well) for 6 days. The stimulatory abilities of allogeneic T cells and allogeneic LGL were tested both before (open) and after (hatched) *in vitro* activation with PHA (0·1 μg/ml) and IL-2 (20%).

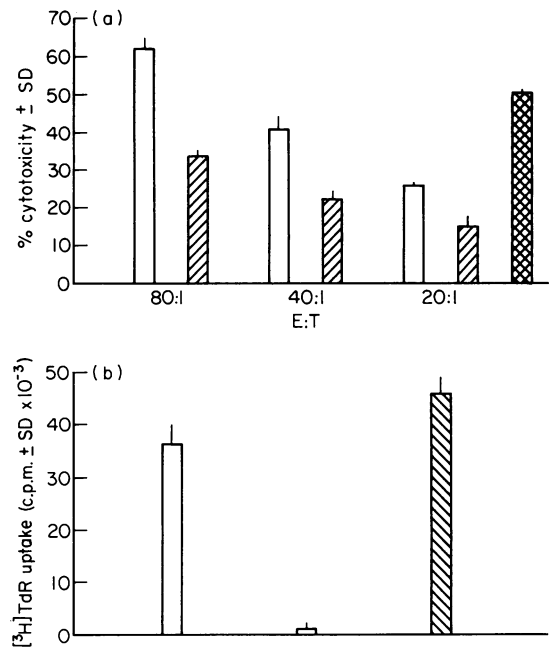

Figure 4. Cytotoxicity of SRBC-rosetting cells. Cytotoxicity against the K562 cell line was measured, at a range of effector : target (E : T) ratios, in nylon-non-adherent (□), SRBC-rosetting (■) and non-SRBC-rosetting (▩) populations (a). The nylon-non-adherent and SRBC-rosetting populations were also assayed for SLO-responsiveness (b). (▧) Denotes reconstitution of SRBC-rosetting cells with 10% monocytes.

incubated in medium without exogenous stimulation. The level of proliferation generated by allogeneic activated cells was generally lower than that generated by allogeneic PBMC.

LGL content of rosetting cell populations

Since LGL have not previously been considered capable of APC function, past investigations requiring the isolation of antigen-non-responsive cell populations have concentrated on the removal of Mo, without consideration of contaminant LGL. Very often, antigen-non-responsive populations have been isolated by either one (Gonwa *et al.*, 1983; Bjercke & Gaudernack, 1985) or two (Scala & Oppenheim, 1983) cycles of SRBC-rosetting. Since LGL are low-affinity SRBC-rosetting cells, it seems likely that they will contaminate T-cell populations isolated in this way. The presence of LGL in non-responsive rosetting cell populations was confirmed by measurement of cytotoxic activity against the NK-sensitive cell line K562 (Fig. 4). Thus, it was found that, although the greater part of the NK activity resided in the non-rosetting population, significant killing of K562 was also a characteristic of the antigen-non-responsive rosetting cells.

Effect of exogenous IL-1 on presentation by LGL

Although LGL have been shown to be capable of producing IL-1 when stimulated with lipopolysaccharide (Scala *et al.*, 1984), in order to be effective in antigen presentation *in vitro*, LGL should produce IL-1 under the conditions of the experimental APC assay employed. In order to determine whether perhaps the absence of IL-1 production by LGL in our assay system might be the reason for the antigen-non-responsiveness of LGL-containing populations, the effect of applying IL-1 exogenously to FACS-sorted cell populations was investigated. Over the concentration range 0–10^3 U/ml, IL-1 only minimally enhanced the capability of Mo to present SLO, and completely failed to induce presentation of SLO by LGL.

DISCUSSION

In order to activate antigen-specific Th, antigen must be presented by APC. This presentation consists of several steps, namely uptake of antigen by the APC, structural alteration or processing of the antigen, re-expression of the antigen on the cell surface in association with Class II molecules, and, additionally, production of the cytokine IL-1. Any cell that is an effective APC must be capable of mediating these steps.

The observation that a subset of LGL is Class II-positive (Ortaldo *et al.*, 1981), coupled with the inducible production of IL-1 by this subset (Scala *et al.*, 1984), has led to the speculation that LGL, in common with other cells possessing these two characteristics, may be effective APC. Indeed, Scala *et al.* (1985) purport to have demonstrated that a subset of human LGL exerts accessory cell activity. Thus, they report that a OKT11+, OKM1+, DR+ subset of LGL can function in an accessory capacity in the induction of T-cell responses to the soluble antigens *Staphylococcus* Protein A and SLO and to cell-surface antigens presented in both an autologous and allogeneic MLR. However, although we have been able to confirm that a subset of LGL does indeed express Class II molecules, no evidence has been produced to support the claim that they are effective APC. In our hands, SLO-induced proliferative responses in human peripheral blood lymphocytes are absolutely dependent on Mo and/or DC, and LGL have no discernible part in the phenomenon.

The lack of concordance between our data and those of Scala *et al.* (1985) is likely to be a consequence of the difference in the LGL-isolation procedures employed. Thus, the LGL used

by Scala *et al.* (1985) in reconstitution experiments were isolated from PBL on the basis of the low buoyant density of LGL, by means of a seven-step Percoll density gradient. In certain of our experiments, LGL-containing T-cell populations were obtained either by reconstitution of T cells with LGL isolated by FACS-sorting with an LGL-specific MoAb, or simply by FACS-sorting unlabelled nylon-non-adherent cells. Confronted with the apparently disparate natures of the LGL populations isolated by these two procedures with respect to APC activity, it may be important to consider the possibility that FACS sorting of LGL affects their functional capacity in some way. Although it is known that FACS-isolated LGL are functional in one respect at least, in that they are enriched for NK activity (Roberts, 1984), it is possible that the FACS sorting procedure renders LGL incapable of APC activity. However, the observation that rosetting cell populations exhibiting NK activity are SLO-non-responsive further implies that LGL do not exhibit APC activity. It is of course possible that the APC activity of LGL is attributable to a subset of LGL which is absent from rosetting cell populations. However, since it has been reported that virtually all of the NK activity of LGL is associated with the Class II-positive subset (Scala *et al.*, 1985), rosetting cell populations exhibiting NK activity should also contain Class II-positive LGL.

A possible criticism of LGL isolation by Percoll density gradient centrifugation is that monocytes present in PBL before fractionation will tend to cofractionate with LGL. This possibility was countered to some extent by Scala *et al.* (1985) by the further purification of LGL-enriched populations by treatment with Leu-M1 (a monocyte-specific MoAb) and complement. However, since DC may be effective APC, in the event that they should have a similar buoyant density to monocytes and also cofractionate with LGL, then contamination of LGL-enriched fractions with DC may be an important factor, since DC will not be removed by complement depletion with monocyte-specific MoAb.

Since a multiplicity of Class II-positive cell types can present antigen (Grey & Chesnut, 1985), it has been speculated that any cell type can perform this function, and that the only special property required for antigen presentation is the expression of Class II molecules. This view is supported by experiments in which transfection of either mouse (Malissen *et al.*, 1984; Norcross *et al.*, 1984) or human (Austin *et al.*, 1985) Class II genes into mouse L cells results in membrane Ia expression, and the concomitant conversion of non-antigen presenting L cells into effective APC. Similarly, when purified Ia antigen is transferred to Ia-negative L cells or B-cell tumours by fusion of Ia-containing liposomes to these cells, the ability to present ovalbumin peptides is also acquired (Grey & Chesnut, 1985), However, there are several examples in the literature of Class II-positive cells that are deficient in APC function. Thus, it has been reported that although both Mo and DC express similar amounts of Class II antigen, only dendritic cells are effective APC (van Voorhis *et al.*, 1983; Bjercke & Gaudernack, 1985). A less controversial example than the monocyte of a cell type whose Class II expression does not confer APC function, is the resting B cell (Frohman & Cowing, 1985). Additionally, not all Class II-expressing tumour cell lines are able to present antigen (Walker *et al.*, 1982), and Class II-positive epithelial cells are restricted in the types of antigen that they can present, a restriction which is compatible with an inability to process antigen (Londei *et al.*, 1984). Although human T cells activated in culture by a variety of agents and expressing surface Class II molecules can function as APC in the generation of mixed lymphocyte reactions (Engleman, Benike & Charron, 1980) and in the induction of both primary (Brown *et al.*, 1984) and secondary (Rich *et al.*, 1980) hapten-specific immune responses, reports regarding their ability to function as APC in the presentation of soluble antigens are contradictory. Thus, although in one paper alloactivated Class II-positive T cells were able to present tetanus and diphtheria toxids to unprimed T cells (Engleman *et al.*, 1980), a more recent investigation concludes that activated human T cells cannot present soluble antigens (Gerrard *et al.*, 1985), and we have also been unable to demonstrate presentation of SLO by PHA- and IL-2-activated T cells. The data presented in this report suggest that the Class II-positive subset of LGL is another example of a Class II-positive cell population that is ineffective in antigen presentation.

It seems to us that the inability of Class II-positive LGL to present antigen could theoretically be attributed to one or more of three factors: (i) the Class II determinants that LGL express may be irrelevant to antigen presentation; (ii) LGL may be incapable of IL-1 production in the experimental system employed in this investigation, or (iii) LGL may be unable to carry out the necessary antigen uptake and processing events. The inability of LGL to present antigen does not appear to be related to a restricted expression of Class II specificities since the expression of the DP, DQ and DR sublocus products by LGL seems to follow a similar pattern to the expression by Mo populations, which are effective APC. In addition, the incubation of LGL and T cells with an exogenous supply of IL-1 does not elicit APC activity by LGL. It thus seems more likely that the deficiency of APC function by LGL is related to (iii), namely the absence of an effective antigen sequestration and/or processing mechanism. This possibility is supported by the ability of highly Class II-positive LGL to present antigen in a situation where processing is not required, namely in an allogeneic MLR.

We have here been unable to confirm the claim that, in addition to exerting NK cytotoxic activity, LGL are important mediators of immunoregulation by mediating the antigen-specific activation of helper T cells. It appears that more specialized cell types that possess certain vital characteristics in addition to expression of Class II antigens are required to generate humoral and cell-mediated responses. However, LGL may still be effective in immunoregulation, both in a general capacity through the production of immunomodulatory lymphokines, and in the specific regulation of haematopoiesis and B-cell activity.

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REFERENCES

ABRUZZO L.V. & ROWLEY D.A. (1983) Homeostasis of the antibody response: immunoregulation by NK cells. *Science*, **222,** 581.

ARAI S., YAMAMOTO H., ITOH K. & KUMAGAI K. (1983) Suppressive effect of human natural killer cells on Pokeweed mitogen-induced B cell differentiation. *J. Immunol.* **131,** 651.

AUSTIN P., TROWSDALE J., RUDD C., BODMER W., FELDMANN M. & LAMB J. (1985) Functional expression of HLA-DP genes transfected into mouse fibroblasts. *Nature (Lond.)*, **313,** 61.

BJERCKE S. & GAUDERNACK G. (1985) Dendritic cells and monocytes as accessory cells in T-cell responses in man. II. Function as antigen-presenting cells. *Scand. J. Immunol.* **21,** 501.

BRIEVA J.A., TARGAN S. & STEVENS R.H. (1984) NK and T cell subsets regulate antibody production by human *in vivo* antigen-induced lymphoblastoid B cells. *J. Immunol.* **132,** 611.

BROWN M.F., VAN M., ABRAMSON S.L., FOX E.J. & RICH R.R. (1984) Cellular requirements for induction of human primary proliferative responses to trinitrophenyl-modified cells. *J. Immunol.* **132,** 19.

CORDELL J.L., FALINI B., ERBER W.N., GHOSH A.K., ABDULAZIZ Z., MACDONALD S., PULFORD K.A.F., STEIN H. & MASON D.Y. (1984) Immunoenzymatic labelling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP complexes). *J. Histochem. Cytochem.* **32,** 219.

ENGLEMAN E.G., BENIKE C.J & CHARRON D.J. (1980) Ia antigen on peripheral blood mononuclear leucocytes in man. II. Functional studies of HLA-DR-positive T cells activated in mixed lymphocyte reactions. *J. exp. Med.* **152,** 114s.

FROHMAN M. & COWING C. (1985) Presentation of antigen by B cells: functional dependence on radiation dose, interleukins, cellular activation and differential glycosylation. *J. Immunol.* **134,** 2269.

GERRARD T.L., VOLKMAN D.J., JURGENSEN C.H. & FAUCI A.S. (1985) Activated human T cells can present alloantigens but cannot present soluble antigens. *Cell Immunol.* **95,** 65.

GONWA T.A., PICKER L.J., RAFF H.V., GOYERT S.M., SILVER J. & STOBO J.D. (1983) Antigen-presenting capabilities of human monocytes correlates with their expression of HLA-DS, an Ia determinant distinct from HLA-DR. *J. Immunol.* **130,** 706.

GREY H.M. & CHESNUT R. (1985) Antigen processing and presentation to T cells. *Immunol. Today*, **6,** 101.

GUY K., VAN HEYNINGEN V., COHEN B.B., DEANE D.L. & STEEL C.M. (1982) Differential expression and serologically distinct subpopulations of human Ia antigens detected with monoclonal antibodies to Ia alpha and beta chains. *Eur. J. Immunol.* **12,** 942.

HANSSON M., KIESSLING R. & ANDERSSON B. (1981) Human fetal thymus and bone marrow contain target cells for natural killer cells. *Eur. J. Immunol.* **11,** 8.

HERBERMAN R.B. (ed.) (1982) *NK Cells and Other Natural Effector Cells.* Academic Press, New York.

VAN HEYNINGEN V., GUY K., NEWMAN R. & STEEL C.M. (1982) Human MHC Class II molecules as differentiation markers. *Immunogenetics*, **16,** 459.

JULIUS M.H., SIMPSON E. & HERZENBERG L.A. (1973) A rapid method for the isolation of functional thymus-derived murine lymphocytes. *Eur. J. Immunol.* **3,** 645.

KASAHARA T., DJEU J.Y., DOUGHERTY S.F. & OPPENHEIM J.J. (1983) Capacity of human large granular lymphocytes (LGL) to produce multiple lymphokines: interleukin 2, interferon and colony stimulating factor. *J. Immunol.* **131,** 2379.

KO H.-S., FU S.M., WINCHESTER R.J., YU D.T.Y. & KUNKEL H.G. (1979) Ia determinants on stimulated human T lymphocytes. Occurrence on mitogen- and antigen-activated T cells *J. exp. Med.* **150,** 246.

LEE K.-C., WONG M. & SPITZER D. (1982) Chloroquine as a probe for antigen processing by accessory cells. *Transplantation*, **34,** 150.

LONDEI M., LAMB J.R., BOTTAZZO G.F. & FELDMANN M. (1984) Epithelial cells expressing aberrant MHC Class II determinants can present antigen to cloned human T cells. *Nature (Lond.)*, **312,** 639.

MAKGOBA M.W., HILDRETH J.E.K. & MCMICHAEL A.J. (1983) Identification of human Ia antigen that is different from HLA-DR and DC antigens. *Immunogenetics*, **17,** 623.

MALISSEN B., PRICE M.P., GOVERMAN J.M., MCMILLAN M., WHITE J., KAPPLER J., MARRACK P., PIERRES A., PIERRES M. & HOOD L. (1984) Gene transfer of H-2 Class II genes: antigen presentation by mouse fibroblast and hamster B-cell lines. *Cell*, **36,** 319.

NORCROSS M.A., BENTLEY D.M., MARGULIES D.H. & GERMAIN R.N. (1984) Membrane Ia expression and antigen-presenting accessory cell function of L cells transfected with Class II major histocompatibility complex genes. *J. exp. Med.* **160,** 1316.

ORTALDO J.R., SHARROW S.O., TIMONEN T. & HERBERMAN R.B. (1981) Determination of surface antigens on highly purified human NK cells by flow cytometry with monoclonal antibodies. *J. Immunol.* **127,** 2401.

PERUSSIA B., ACUTO O., TERHORST C., FAUST J., LAZARUS R., FANNING V. & TRINCHIERI G. (1983) Human natural killer cells analysed by B73.1, a monoclonal antibody blocking Fc receptor functions. II. Studies of B73.1 antibody–antigen interaction on the lymphocyte membrane. *J. Immunol.* **130,** 2142.

PISTOIA V., COZZOLINO F., TORCIA M., CASTIGLI E. & FERRARINI M. (1985) Production of B cell growth factor by a Leu-7$^+$, OKM1$^+$ non-T cell with the features of large granular lymphocytes (LGL). *J. Immunol.* **134,** 3179.

RAFF H.V., PICKER L.J. & STOBO J.D. (1980) Macrophage heterogeneity in man. A subpopulation of HLA-DR bearing macrophages required for antigen-induced T cell activation also contains stimulators for autologous-reactive T cells. *J. exp. Med.* **152,** 581.

REINHERZ E.L., KUNG P.C., PESANDO J.M., RITZ J., GOLDSTEIN G & SCHLOSSMAN S.F. (1979) Ia determinants on human T-cell subsets defined by monoclonal antibody. Activation stimuli required for expression. *J. exp. Med.* **150,** 1472.

RICH R.R., ABRAMSON S.L., SELDIN M.F., PUCK J.M. & LEVY R. (1980) Role of Ia-positive cells in induction of secondary human immune responses to haptens *in vitro*. *J. exp. Med.* **152,** 218s.

ROBERTS K. (1984) Human natural killer cells—a clonal analysis. *PhD Thesis.* Victoria University of Manchester.

ROBERTS K. & MOORE M. (1985) A clonal analysis of human peripheral blood lymphocytes displaying natural killer-like activity. *Eur. J. Immunol.* **15,** 448.

SCALA G., ALLAVENA P., DJEU J.Y., KASAHARA T., ORTALDO J.R., HERBERMAN R.B. & OPPENHEIM J.J. (1984) Human large granular lymphocytes are potent producers of interleukin-1. *Nature (Lond.)*, **309,** 56.

SCALA G., ALLAVENA P., ORTALDO J.R., HERBERMAN R.B. & OPPENHEIM J.J. (1985) Subsets of human large granular lymphocytes (LGL) exhibit accessory cell functions. *J. Immunol.* **134,** 3049.

SCALA G. & OPPENHEIM J.J. (1983) Antigen presentation by human monocytes: evidence for stimulant processing and requirement for interleukin 1. *J. Immunol.* **131,** 1160.

STEEL C.M. (1984) Table of workshop monoclonal antibodies and a synopsis of their principal characteristics. *Disease Markers*, **2,** 363.

SUNDERLAND C.A., NAIEM M., MASON D.Y., REDMAN C.W.G. & STIRRAT G.M. (1981) The expression of major histocompatibility antigens by human chorionic villi. *J. Reprod. Immun.* **3,** 323.

TILDEN A.B., ABO T. & BALCH C.M. (1983) Suppressor cell function of human granular lymphocytes identified by the HNK-1 (Leu 7) monoclonal antibody. *J. Immunol.* **130,** 1171.

TIMONEN T., ORTALDO J.R. & HERBERMAN R.B. (1981) Characteristics of human large granular lymphocytes and relationship to natural killer and K cells. *J. exp. Med.* **153,** 569.

TIMONEN T., RANKI A., SAKSELA E. & HÄYRY P. (1979a) Human natural cell-mediated cytotoxicity against fetal fibroblasts. III. Morphological and functional characterisation of effector cells. *Cell Immunol.* **48,** 121.

TIMONEN T., SAKSELA E., RANKI A. & HÄYRY P. (1979b) Fractionation, morphological and functional characterisation of effector cells re-

sponsible for human natural killer activity against cell-line targets. *Cell Immunol.* **48,** 133.

UNANUE E.R. (1984) Antigen-presenting function of the macrophage. *Ann. Rev. Immunol.* **2,** 395.

VAN VOORHIS W.C., VALINSKY J., HOFFMAN E., LUBAN J., HAIR L.S. & STEINMAN R.M. (1983) Relative efficacy of human monocytes and dendritic cells as accessory cells for T cell replication. *J. exp. Med.* **158,** 174.

WALKER E., WARNER N., CHESNUT R., KAPPLER J. & MARRACK P. (1982) Antigen-specific, I region-restricted interactions *in vitro* between tumour cell lines and T cell hybridomas. *J. Immunol.* **128,** 2164.

WEINER M.S., BIANCO C. & NUSSENZWEIG V. (1973) Enhanced binding of neuraminidase-treated sheep erythrocytes to human lymphocytes. *Blood,* **42,** 939.

YAM L.T., LI C.Y. & CROSBY W.H. (1971) Cytochemical identification of monocytes and granulocytes. *Am. J. clin. Pathol.* **55,** 283.

ZEMBALA M., URACZ W., RUGGIERO I., MYTAR B. & PRYJMA J. (1984) Isolation and functional characteristics of FcR^+ and FcR^- human monocyte subsets. *J. Immunol.* **133,** 1293.

ZIEGLER A., UCHANSKA-ZIEGLER B., ROSENFELDER G., BRAUN D.G. & WERNET P. (1981) Heterogeneity of established human hematopoietic cell lines: surface antigens detected by monoclonal antibodies and glycosphinglopid patterns. In: *Leukaemia Markers,* Proceedings of the Leukaemia Marker Conference held in Vienna, February 1981, p. 317. Academic Press, New York.