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Gamma-interferon production by human low-density lymphocytes induced by T-cell mitogens

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SUMMARY

Low-density lymphocytes prepared by Percoll fractionation of human peripheral blood mononuclear leucocytes were found to produce large amounts of interferon-γ (IFN-γ) in response to different T-cell mitogens in the absence of macrophages, whilst higher density lymphocytes were strongly dependent on the presence of macrophages for significant IFN-γ production. The addition of macrophages to the low-density lymphocytes made little difference to their IFN-γ production. Subsets of the low-density lymphocytes prepared by rosetting with sheep red blood cells produced markedly less IFN-γ than did the original population; IFN-γ production could be largely restored by recombining the two low-density fractions. This suggests that IFN-γ production by low-density lymphocytes whilst macrophage-independent does require co-operation between different cell types. The low-density lymphocytes were enriched for cells bearing the Leu 11 and OKM1 antigens, and for natural killer cell activity. The rosetting fraction was enriched for OKT3 antigen-bearing cells, and the non-rosetting fraction was enriched for Leu 11, OKM1 antigen-bearing cells. Depletion of B cells (surface Ig-positive) by nylon-wool chromatography had no effect on IFN-γ production by low-density lymphocytes.

INTRODUCTION

A variety of regulatory molecules called lymphokines have been discovered, which appear to regulate the actions of various cell types that participate in the immune response (Marx, 1983). Activated macrophages secrete interleukin-1 (IL-1) which, in turn, induces production of interleukin-2 (IL-2) (Bendtzen & Petersen, 1984; Williams *et al.*, 1984) by activated T cells. IL-2 stimulates T-cell proliferation and interferon-γ (IFN-γ) production (Torres, Farrar & Johnson, 1982; Kasahara *et al.*, 1983b; Croll, Wilkinson & Morris, 1985). A variety of T-cell types have been found capable of producing IFN-γ (Archer *et al.*, 1979), but probably all require the presence of accessory macrophages (Mo) for significant IFN-γ production (Bendtzen & Petersen, 1984; Bruszewski *et al.*, 1984; Chang *et al.*, 1982; Unanue *et al.*, 1984). There is evidence to suggest that non-T cells may also produce IFN-γ. In particular, it has been shown that human low-density lymphocytes produce IFN-γ and a variety of other lymphokines, including IL-1, IL-2, colony-stimulating factor, and B-cell growth factor, in response to T-cell mitogens (Kasahara *et al.*, 1983a; Djeu, 1983; Scala *et al.*, 1984; Pistoia *et al.*, 1985). This IFN-γ production is often ascribed to large granular lymphocytes (LGL), which make up a large proportion of the low-density fraction and possess natural killer cell characteristics. Removal of T cells from these heterogeneous fractions by treatment with the monoclonal antibody OKT3 plus complement increased both IL-1 (Scala *et al.*, 1984) and IL-2 production, but slightly reduced IFN-γ production (Kasahara *et al.*, 1983a). LGLs, which make up less than 5% of the total peripheral blood mononuclear leucocytes, have a distinct morphology of high cytoplasmic to nuclear ratio with a granular cytoplasm. Nearly all natural killer cell activity can be attributed to the LGL population (Timonen, Ortaldo & Herberman, 1981), and they bear the surface markers Leu 7, Leu 11 and OKM1 (Scala *et al.*, 1985; Abo, Miller & Balch, 1984).

In this paper, we have studied IFN production by low-density lymphocytes in response to the T-cell mitogens PHA, Con A and SEA. We find that the low-density lymphocytes do not require macrophages for IFN-γ production, unlike high-density T lymphocytes. However, this IFN production appears partially dependent upon the interaction of two subsets separable by sheep red blood cell rosetting. The two subsets are enriched for OKT3$^+$ lymphocytes and Leu 11 and OKM1$^+$ lymphocytes.

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Abbreviations: Con A, concanavalin A; IFN-γ, interferon-γ; IL-1, interleukin-1; IL-2, interleukin-2; LGL, large granular lymphocytes; PBML, peripheral blood mononuclear leucocytes; PHA, phytohaemagglutinin; SEA, *Staphylococcus aureus* enterotoxin A; SRBC, sheep red blood cells.

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MATERIALS AND METHODS

Cell preparation

Peripheral blood mononuclear leucocytes (PBML) were obtained by centrifugation over Ficoll–Hypaque (Pharmacia Ltd, Milton Keynes, Bucks) of human buffy coat residues from normal blood (provided by the U.K. West Midlands Blood Transfusion Centre, Edgbaston, Birmingham). The cells were cultured at 5×10^5 cells/ml in RPMI-1640 medium buffered with HEPES and supplemented with 10% fetal calf serum (Gibco Ltd, Paisley, Renfrewshire), penicillin (60 μg/ml) and streptomycin (100 μg/ml).

Macrophages were recovered from PBML by adherence to plastic petri-dishes with repeated washings to remove non-adherent cells. Adherent cells were removed by incubation with phosphate-buffered saline containing 5% fetal calf serum (FCS) and 0·2% EDTA at 4° for 45 min. The addition of indian ink to recovered cells showed them to be 99% phagocytic in nature.

Macrophage-depleted lymphocytes were prepared by replating PBML three times at hourly intervals on plastic petri-dishes to remove adherent cells. Silica was then added at 200 μg/ml overnight to eliminate the few remaining phagocytic cells. The lymphocytes were then isolated over Ficoll at 350 ***g*** to remove the silica and cell debris. This method produced >99% pure lymphocyte preparations as judged by non-specific esterase staining.

Separation of lymphocytes into differing density subpopulations was achieved by centrifugation at 550 ***g*** for 30 min over a six-step discontinuous Percoll (Pharmacia Ltd) RPMI gradient, the steps of which varied by 2·5% Percoll concentrations from 40 to 52·5% Percoll. The Percoll was buffered by mixing eight parts of 10× concentrated phosphate-buffered saline with 92 parts Percoll, before adding RPMI-1640/10% FCS for the required concentrations. Cells were harvested from each layer and counted to determine the percentage of total lymphocyte population present per step. Cells from the top one to three steps were used as the low-density fraction, varying from 5 to 30% of the total lymphocyte population, the percentage depending on the efficiency of separation. The low-density lymphocytes were chiefly found at the interface between the 42·5% and 45% steps. In later experiments a single 46% Percoll cushion was used to isolate low-density cells. Cells isolated from the bottom three steps or below the 46% interface were considered high density, chiefly T cells.

The low-density population was further fractionated into E^+ and E^- cells by high-affinity sheep red blood cell (SRBC) rosetting (Timonen *et al.*, 1981), which separates T from non-T cells by virtue of T cells bearing a cell surface antigen called the sheep erythrocyte or E receptor. Some subsets of non-T cells also possess an E receptor (Timonen *et al.*, 1981), but it is of low affinity for sheep erythrocytes compared to that of T cells. Briefly, lymphocytes were washed twice in PBS and resuspended at 2×10^6 cells/ml. SRBC, after three PBS washes, were resuspended in medium at 3×10^8 cells/ml. Equal volumes of lymphocytes and erythrocytes were mixed and an equal volume of FCS added. After gentle pelleting (200 ***g***), the cells were incubated at 29° for 1 hr. After gentle resuspension with a wide-bore pipette, the rosetted and non-rosetted cells were separated by Ficoll centrifugation. SRBC were removed from rosetted lymphocytes by incubation in lysis buffer (0·14 M ammonium chloride, 20 mM Tris, pH 7·5) for 10 min at 37°, followed by three washes in buffer.

An alternative method for the removal of both adherent macrophages and B lymphocytes was by nylon-wool chromatography (Julius, Simpson & Herzenberg, 1973). PBML (5×10^7 cells/ml) in RPMI (2% FCS) was passed through a prewarmed (37°) nylon-wool column (0·6 g nylon wool/10^8 cells) and incubated at 37° for 45 min. Non-adherent cells were gently eluted with medium, leaving adherent macrophages and B cells in the column. This non-adherent population could then be further treated with adherence and silica to produce very pure lymphocyte populations depleted of both macrophages and B lymphocytes.

Cell culture and mitogens

The various cell fractions were incubated at 5×10^5 cells/ml in 200-μl well (round-bottomed) microtitre plates (Flow Labs Ltd, Uxbridge, Middlesex). When macrophages were added back (from the same donor), a lymphocyte:macrophage ratio of 10:1 was used since this is similar to that normally found and gives the best enhancement of IFN production. The cells were incubated for 3 days at 37° before removing supernatant samples for IFN assay.

Purified *Staphylococcus aureus* enterotoxin A (SEA), supplied by the Food and Drug Administration of the USA (Washington, DC), was the mitogen chiefly used since it is a potent inducer of IFN production. Other mitogens used were purified phytohaemagglutinin (PHA) from Wellcome Research Laboratories (Beckenham, Kent) and concanavalin A (Con A) from Miles-Yeda Ltd (Slough, Berks). These mitogens were used at concentrations previously shown to be optimal for IFN-γ induction: SEA (20 ng/ml), Con A (20 μg/ml) and PHA (10 μg/ml).

IFN assay

Interferon, in 3-day post-induction supernatants, was assayed by the $INAS_{50}$ method (Atkins *et al.*, 1974) using Semliki Forest virus to challenge human amnion (WISH) cells. Viral replication was measured by tritiated uridine incorporation, the extent of which depended on the degree to which an IFN sample had induced an anti-viral state. A laboratory IFN-γ standard was included to calibrate the assays, and this was itself calibrated against an international standard of human reference IFN-γ (National Institutes of Health, Bethesda, MD). Laboratory units of IFN were found to be equivalent to reference units. A two-fold difference in titre between two samples of IFN was considered to be statistically significant since in a series of replicate titrations of the same standard sample 95% of the titres obtained lay within a two-fold range.

Immunofluorescence and staining techniques

Indirect immunofluorescence was used to characterize the cell fractions using an initial incubation with a murine monoclonal antibody and a second incubation with fluorescein (FITC)-labelled rabbit anti-mouse Ig. Monoclonal antibodies OKT3 and OKM1 came from Ortho Diagnostic Systems Inc. (Raritan, NJ) and Leu 11 from Becton-Dickinson (Mountain View, CA). The latter required an IgM-specific FITC or rhodamine (RITC) conjugate. An IgG-specific FITC conjugate was used for the Ortho monoclonal antibodies.

In order to determine the percentage of B cells (surface Ig^+), cells were incubated with FITC-labelled rabbit anti-human IgG.

The presence of macrophages was determined by the

Table 1. Production of IFN by lymphocytes of different densities in response to different mitogens

Cell fraction	IFN titre (U/ml)					
	SEA		Con A		PHA	
	Alone	With Mo	Alone	With Mo	Alone	With Mo
Lymphocytes	50	2000	< 8	130	16	50
Macrophages	< 8		< 8		< 8	
Fraction 1 (3%)	800	130	200	250	32	32
Fraction 2 (3%)	400	500	250	250	160	250
Fraction 3 (27%)	80	200	20	160	20	20
Fraction 4 (53%)	40	200	13	160	< 8	25
Fraction 5 (14%)	20	200	< 8	80	< 8	20

All cell populations were incubated at 5×10^5 cells/ml and the IFN titre measured 3 days after induction with SEA (20 ng/ml). Con A (20 μg/ml), or PHA (10 μg/ml). Those fractions where macrophages were added back, at 1:10 concentration, are indicated in the columns labelled 'With Mo'. Fraction 1 contained the lowest density cells, and Fraction 5 the highest. The figure in parentheses represent the percentage of the total lymphocyte population found in each Percoll layer.

addition of indian ink. A 1-hr incubation with 0·5% indian ink followed by microscopic examination revealed the presence of any phagocytic cells. A non-specific esterase-staining kit (Sigma, Poole, Dorset) was also used to test for the presence of macrophages, the enzyme being macrophage/monocyte specific.

RESULTS

Low-density lymphocytes are high-level producers of IFN-γ independent of macrophages

When macrophage-depleted lymphocytes were separated on a stepped gradient and treated with different mitogens (SEA, Con A, PHA), it can be seen in Table 1 that the low-density lymphocytes from Fractions 1 and 2, comprising 6% of the total lymphocytes, were the best IFN producers on a per cell basis. The interferon produced was shown to be IFN-γ for both total lymphocytes and low-density cells by its 99% neutralization with anti-IFN-γ antiserum (data not shown). The addition of macrophages made little difference to IFN-γ production by low-density cells, but boosted IFN-γ production five- to 10-fold for the denser and total lymphocytes. This was the case for all three mitogens and for cells from several different donors.

In other experiments, when total lymphocyte populations were reconstituted from low- and high-density cells, the requirement for macrophages was restored to that found in the unfractionated lymphocyte population. Thus, only low-density lymphocyte fractions are macrophage-independent. The removal of B lymphocytes by nylon-wool chromatography does not alter the observation that only the low-density cells can produce significant IFN levels without macrophages. Therefore, B lymphocytes are not involved in IFN production by low-density cells.

Co-operation between subsets of low-density lymphocytes is necessary for maximal IFN-γ production

When the low-density lymphocytes were further fractionated by high-affinity SRBC rosetting into E^+ and E^- cells, the former bearing the sheep erythrocyte receptor, the IFN-γ production of these two populations on a per cell basis was lower than that of unfractionated low-density cells (Table 2), with the non-rosetting fraction generally producing more IFN than the

Table 2. IFN production by subfractions of low-density cells

Cell fraction	IFN titres (U/ml) Donors				
	1	2	3	4	5
PBML	800	320	400	200	320
Lymphocytes	260	80	120	8	75
Low density	500	400	320	5000	205
Low-density E^-	250	120	100	40	105
Low-density E^+	160	5	55	18	125
Reconstituted E^-/E^+	320	220	225	40	325

Five experiments were carried out with PBML from different donors. All cell populations except the PBML had been depleted of macrophages. Cell concentration was 5×10^5 cells/ml, and 20 ng/ml SEA was used as inducer in all cases. The reconstituted low-density population was a mixture of E^- and E^+ cells in the proportions found after rosetting the low-density cells.

Table 3. Macrophage assistance of low-density cell subfractions in IFN production

	IFN titres (U/ml)					
	Donors					
	1		2		3	
Cell fraction	Alone	With Mo	Alone	With Mo	Alone	With Mo
Lymphocytes	20	160	80	800	6	2000
Low density	100	400	400	350	1100	3200
Low-density E^-	32	170	120	600	60	1600
Low-density E^+	3	100	5	400	50	450
Reconstituted E^-/E^+	ND		220	110	ND	

Three experiments were carried out with cells from different donors. All cell populations were at 5×10^5 cells/ml and induced with 20 ng/ml SEA. Macrophages were added back at a 1:10 ratio (macrophage to lymphocyte) to those fractions in the columns labelled 'With Mo'.

rosetting fraction. In order to check whether this reduction of IFN production by the subfractions was due to cell damage during the fractionation procedures, reconstitution experiments were carried out. It was found that reconstitution of the low-density population by mixing E^+ and E^- cells largely restored IFN production to its unfractionated level (Table 2).

In additional experiments, macrophages were added to the E^+ and E^- fractions and the IFN production measured. The addition of macrophages to E^+ or E^- cells boosted IFN levels, especially in the case of E^+ cells (Table 3).

Characterization of cell fractions

In order to characterize the various cell fractions, the expression of three leucocyte surface markers (Leu 11, OKT3 and OKM1) by the cells was examined by immunofluorescence. Leu 11 is a marker found on LGLs, OKT3 is found on T cells, and OKM1 is found on macrophages and some null (i.e. non-T) cells. Unfractionated lymphocytes were 11% Leu 11^+ and 84% $OKT3^+$ (Table 4). A typical low-density preparation was 23% Leu 11 and 38% $OKT3^+$, showing enrichment for the LGL marker. If only the top two steps of the gradient were used for the low-density fraction, preparations containing up to 70% Leu 11^+ cells could be obtained, but the third step was usually included to provide sufficient cells for all the fractionation procedures. After rosetting, the E^- fraction was somewhat enriched for Leu 11^+ cells and the E^+ fraction strongly enriched for T cells. Using different coloured conjugates specific for Leu 11 and OKM1 (fluorescein and rhodamine), the expression of both markers on the same cell was examined. OKM1 was generally coexpressed with Leu 11. A typical low-density fraction contained 42% Leu $11^+/OKM1^+$ cells, but after rosetting the E^+ fraction contained 10% Leu $11^+/OKM1^+$ cells and the E^- fraction contained 60% Leu $11^+/OKM1^+$ with 5% $OKM1^+$ only cells. Thus, the low-density fraction is enriched for the LGL marker Leu 11 and depleted somewhat of T cells. Subsequent rosetting leads to a Leu 11-enriched E^- fraction and an OKT3-enriched E^+ fraction.

Table 4. Cell surface markers of the cell fractions

	% cells bearing marker	
Cells	Leu 11^+	$OKT3^+$
Total lymphocytes	11	84
Low-density lymphocytes	23	38
E^- fraction	50	36
E^+ fraction	7	79

OKM1 was generally coexpressed with Leu 11. The above results come from a typical fractionation of lymphocytes from a single donor.

Non-specific esterase staining to detect cells of the monocyte/macrophage lineage and the addition of indian ink to detect phagocytic cells both confirmed macrophage depletion. Lymphocytes and low-density fractions both contained less than 0·2% macrophages.

NK activity was measured in an 18-hr ^{51}Cr-release assay using K562 target cells at an effector:target ratio of 30:1. Low-density cells were found to be 82% cytotoxic and high-density cells 16% cytotoxic, showing clear enrichment for NK activity in the low-density fraction.

The low-density lymphocyte fraction contained up to 20% SIg^+ cells; this was reduced to less than 2% by nylon-wool chromatography.

DISCUSSION

There have been frequent demonstrations that the induction of IFN-γ by antigens or mitogens in unfractionated lymphocytes is macrophage-dependent. We have found that low-density lym-

phocytes prepared by Percoll fractionation of macrophage-depleted human peripheral blood mononuclear cells did not require macrophages for IFN-γ production, unlike high-density lymphocytes which make up the majority of lymphocytes and are strongly macrophage-dependent. There are two possible explanations for this phenomenon. Either cells in the low-density lymphocyte fractions are able to make IFN-γ independent of the accessory cell function supplied by macrophages, or else there are non-macrophage accessory cells present in the cell fraction, interacting with the remaining T cells also present in the low-density population.

In our studies, we have subfractionated low-density lymphocytes by high-affinity SRBC rosetting. The E^+ subfraction produced in this way consisted largely of $OKT3^+$ lymphocytes and produced little IFN-γ unless macrophages were added back. The E^- subfraction, which contains most of the Leu 11^+, $OKM1^+$ cells but also significant numbers of $OKT3^+$ lymphocytes, produced markedly less IFN-γ than the unfractionated low-density cells, but rather more than the E^+ cells. When the low-density lymphocyte population was reconstituted by mixing together E^+ and E^- cells, IFN-γ production returned to near the level observed with unfractionated low-density lymphocytes. We conclude that some form of co-operation in IFN-γ production takes place between two low-density cell types separable by rosetting. The identities of the cell types involved in IFN-γ production by the low-density lymphocytes is not clear. There is ample evidence from studies with T-cell clones to suggest that T-cells can produce IFN-γ (Matsuyama *et al.*, 1982; Morris, Lin & Askonas, 1982; Pasternak, Bevan & Klein, 1984), and some evidence to indicate that Leu 11^+, $OKM1^+$ cells may also be capable of IFN-γ production. In our experiments, the E^+ cell population consisted chiefly of $T3^+$ cells which were macrophage-dependent for maximal IFN-γ production. The E^- population was enriched for Leu 11^+ cells but also contained a proportion of $T3^+$ cells, and can make significant quantities of IFN-γ in the absence of macrophages. We speculate that Leu 11^+ cells may themselves produce IFN-γ independent of accessory cells, but in addition can act as accessory cells for the production of IFN-γ by low-density T cells. This explains the co-operation for maximal IFN-γ production we observed between the E^+ and E^- low-density lymphocyte.

Production of IL-1, which is probably involved in activation of IFN-γ production (Croll & Morris, manuscript in preparation), and accessory cell function have been ascribed to cells expressing OKM1, DR and B73.1 (equivalent to Leu 11), which would correspond to our E^- subset (Scala *et al.*, 1984). Interaction between subsets of low-density lymphocytes has also been shown to be important for the optimal induction of autologous mixed lymphocyte reactions (Scala *et al.*, 1985). There thus seems to be accumulating evidence for a cell type which, although it shares at least one marker (OKM1) with macrophages, is distinct from macrophages (i.e. non-adherent, non-phagocytic, esterase⁻, Leu 11^+) but able to carry out accessory function. Whether this cell type is able to replace macrophages in other immune responses is as yet unknown. In our experiments the E^- subset of low-density lymphocytes, unlike macrophages, was unable to 'help' high-density lymphocytes to make IFN-γ (data not shown). Hence, the two cell types, at least in this instance, are not interchangeable. However, to demonstrate cell population interaction unambiguously, more homogeneous cell fractions must be prepared and mixed.

Three points emerge from our results. Firstly, our rigorous removal of macrophages demonstrates that low-density lymphocytes, unlike high-density lymphocytes, can make IFN-γ in the absence of macrophages. Secondly, in the absence of macrophages, more than one cell type is necessary for optimal IFN-γ production by low-density cells. Finally, whilst many authors ascribe IFN-γ production by low-density cells to LGLs, we suggest that other mechanisms may exist and consider that low-density T cells may also produce IFN if $OKM1^+$, Leu 11^+ cells act as accessory cells.

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REFERENCES

Abo T., Miller C.A. & Balch C.M. (1984) Characterisation of human granular lymphocyte subpopulations expressing HNK-1 (Leu-7) and Leu 11 antigens in the blood and lymphoid tissues from fetuses, neonates and adults. *Eur. J. Immunol.* **14**, 616.

Archer D.L., Smith B.G., Ulrich J.T. & Johnson H.M. (1979) Immune interferon induction by T-cell mitogens involves different T-cell subpopulations. *Cell. Immunol.* **48**, 420.

Atkins G.J., Johnson M.D., Westmacott L.M. & Burke D.C. (1974) Induction of IFN in chick cells by temperature sensitive mutants of Sindbis virus. *J. gen. Virol.* **25**, 381.

Bendtzen K. & Petersen J. (1984) Role of monocytes/macrophages and interleukin 1 in antigen induced human lymphokine production. *Cell. Immunol.* **83**, 101.

Bruszewski W.B., Bruszewski J.A., Tonnu H., Ferezy S.L., O'Brien R.L. & Parker J.W. (1984) Early mitogen-induced metabolic events essential to proliferation of human T lymphocytes: dependence of specific events on the influence of adherent accessory cells. *J. Immunol.* **132**, 2837.

Chang T.W., Testa D., Kung P.C., Perry L., Dreskin H.J. & Goldstein G. (1982) Cellular origin and interactions involved in IFN-γ production induced by OKT3 monoclonal antibody. *J. Immunol.* **128**, 585.

Croll A.D., Wilkinson M.F. & Morris A.G. (1985) Interleukin 2 receptor blockade by anti-Tac antibody inhibits IFN-γ induction. *Cell. Immunol.* **92**, 184.

Djeu J.Y. (1983) Production of interferon by natural killer cells. *Clin. Immunol. Allergy*, **3**, 561.

Julius M.H., Simpson E. & Herzenberg L.A. (1973) A rapid method for the isolation of functional thymus-derived mouse lymphocytes. *Eur. J. Immunol.* **3**, 645.

Kasahara T., Djeu J.Y., Dougherty S.F. & Oppenheim J.J. (1983a) Capacity of human large granular lymphocytes (LGL) to produce multiple lymphokines: IL2, IFN and Colony Stimulating Factor. *J. Immunol.* **131**, 2379.

Kasahara T., Hooks J.J., Dougherty S.F. & Oppenheim J.J. (1983b) IL2 mediated immune interferon production by human T cells and T cells subsets. *J. Immunol.* **130**, 1784.

Marx J.L. (1983) Chemical signals in the immune system. *Science*, **221**, 1362.

Matsuyama M., Sugamura K., Kawade Y. & Himima Y. (1982) Production of immune interferon by human cytotoxic T-cell clones. *J. Immunol.* **129**, 450.

Morris A.G., Lin Y.-L. & Askonas B.A. (1982) Immune interferon release when a cloned cytotoxic T-cell line meets its correct influenza-infected target cell. *Nature (Lond.)*, **295**, 150.

Pasternack M.S., Bevan M.J. & Klein J.R. (1984) Release of discrete

interferons by cytotoxic T lymphocytes in response to immune and non-immune stimuli. *J. Immunol.* **133,** 277.

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.

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.

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

TORRES B.A., FARRAR W.L. & JOHNSON H.M. (1982) IL2 regulates immune IFN production by normal and suppressor cell cultures. *J. Immunol.* **128,** 2217.

UNANUE E.R., BELLER D.I., LU C.Y. & ALLEN P.M. (1984) Antigen presentation: comments on its regulation and mechanism. *J. Immunol.* **132,** 1.

WILLIAMS J.M., RANSIL B.J., SHAPIRO H.M. & STROM T.B. (1984) Accessory cell requirement for activation antigen expression and cell cycle progression by human T lymphocytes. *J. Immunol.* **133,** 2986.