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Microbicidal activity of monocyte derived macrophages in AIDS and related disorders

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SUMMARY

We have examined the ability of monocyte-derived macrophages from patients with AIDS and other HIV-related disorders to kill the intracellular pathogen *Toxoplasma gondii*. We have also examined the capacity of peripheral blood mononuclear cells from these patients to produce macrophage-activating and other lymphokines. The capacity to produce interleukin 2 and gamma interferon decreases from controls through asymptomatic seropositive subjects and lymphadenopathy groups A (benign) and B (prodromal) to AIDS. The decrease did not correlate precisely with the decrease in $CD4^+$ cells in these patients. Monocyte-derived macrophages from asymptomatic HIV-infected subjects and lymphadenopathy patients showed a decreased ability to kill *T. gondii* after activation with recombinant gamma interferon; paradoxically, this was most striking for PGL group A. The defect was largely overcome by using Concanavalin A stimulated autologous supernatants. It was notable that macrophages from AIDS patients showed normal killing with recombinant gamma interferon, but that the supernatants from AIDS patients had reduced activity with normal macrophages. These studies confirm that functional defects of both lymphocytes and macrophages are found in HIV-infected subjects; they serve to emphasize the heterogeneity of the clinical and biological responses to this retrovirus, responses which have important implications in the pathogenesis and treatment of the immunodeficiency.

Keywords AIDS monocyte-derived macrophages γ-IFN MAF IL-2

INTRODUCTION

One of the major clinical features of the acquired immune deficiency syndrome (AIDS) is presentation with severe opportunist infections which in other clinical settings have long been recognized as indicators of a profound deficiency in cellular immunity. The cause of this immunodeficiency is a human lymphotropic retrovirus designated HIV (Gallo *et al.*, 1983; Barre-Sinoussi *et al.*, 1983; Pinching & Weiss, 1986). An essential component of the receptor for this virus has been shown to be the CD4 antigen expressed on the T helper/inducer population of lymphocytes (Klatzman *et al.*, 1984a, b; Dalgleish *et al.*, 1984). Since this antigen is also expressed on peripheral blood monocytes (Wood, Warner & Warnke, 1983; Moscicki *et al.*, 1983), these and related cells of the macrophage lineage may also be infected in AIDS. Indeed, direct infection of the human promonocyte cell line U937 has been achieved *in vitro* (Dalgleish *et al.*, 1984). Also, defective monocyte function has been shown in AIDS and AIDS-related disease (Pinching *et al.*, 1983; Poli *et al.*, 1985). Thus it is possible that both lymphocytes and monocytes may be infected by the AIDS

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virus; collaboration between these two cell types is essential for the elimination of the facultative intracellular pathogens that are responsible for many of the opportunist infections seen in AIDS. In order to investigate this further, we have used an assay system which allows us to examine both the ability of lymphocytes to produce macrophage-activating lymphokines and the ability of monocyte-derived macrophages to kill the intra-cellular pathogen *Toxoplasma gondii*, one of the important opportunist pathogens found in AIDS.

MATERIALS AND METHODS

Patients. Sixteen patients with AIDS were studied; six presented with Kaposi's sarcoma alone (KS), eight with a history of opportunistic pathogens (OI) and two with both KS and OI. Four of the patients with OI were well at the time of study. All the patients were positive for antibodies to HIV (HIV +ve).

Eighteen patients with persistent generalized lymphadenopathy (PGL) were studied. Nine of these patients exhibited a benign non-progressive form of lymphadenopathy (PGL-A) and nine had a subtle progressive form showing signs of immunodeficiency (PGL-B or 'prodromal AIDS'; Pinching, 1985). Only one member of this group of 18 patients was found to be HIV −ve, and was presumably a 'false negative'.

A group of asymptomatic, sexually active, homosexual men (AH) were recruited from a well documented cohort attending the Praed Street Clinic at St Mary's Hospital. Half of this group of 34 individuals was HIV +ve.

A control group of healthy heterosexual males was recruited from laboratory personnel.

Lymphocyte studies. The total number of peripheral blood T cells, T helper cells (T4, $CD4^+$) and T suppressor/cytotoxic cells (T8, $CD8^+$) were determined on an EPICS V fluorescence activated cell sorter using the monoclonal antibodies Leu 4, Leu 3a and Leu 2a (Becton Dickinson), as previously described (Pinching *et al.*, 1984).

Monocyte culture. Peripheral blood mononuclear cells (PBMC) obtained by density flotation over Lymphoprep (Nyegaard) were washed three times and resuspended to 2×10^6 cells/ml in RPMI 1640 supplemented with glutamine (2%), penicillin (100 U/ml), streptomycin (100 μg/ml), and 30% heat inactivated human AB serum (RPMI ab). At this stage no statistical difference in cell viability or in the proportion of monocytes was observed between the control and patient groups. Two hundred and fifty microlitre aliquots of the cells were incubated in round-bottomed, 96-well microtitre plates for 2 h (37°C) to allow adherence of monocytes. Non-adherent cells were removed by extensive washing and 200μl of medium was added to each well. After 24 h incubation (37°C; 5% CO_2) the medium was replaced and the plates cultured for a further 6 days. At this stage the cells expressed characteristics of resting macrophages in that they were unable to kill *T. gondii* without prior stimulation (Anderson & Remington, 1974).

Measurement of Toxoplasma *killing.* A modification of the method of McLeod & Remington (1979) was used to assay macrophage killing of *T. gondii*. Briefly, *T. gondii* (RH strain) were passaged in BALB/c mice and adjusted to 5×10^6organisms/ml in RPMI. Half the wells containing macrophages cultured for 7 days were stimulated with either recombinant γ-IFN (rγ-IFN; 200 U/ml; Biogen, Switzerland) or with autologous or heterologous Concanavalin A (Con A, Sigma) stimulated supernatant (20% v/v). After 24 h, half the stimulated and unstimulated wells were supplemented with 10 μl of the *Toxoplasma* suspension. The plates were incubated for 24 h. One microcurie of tritiated uracil (^{3}H-U) was added to each well for 18 h. The plates were then repeatedly frozen and thawed, and harvested using a Skatron AS cell harvester. ^{3}H-U incorporation was evaluated by liquid scintillation using a Packard Tri-carb β counter. This technique did not allow the direct determination of phagocytosis. However, this was performed using light microscopy in a pilot study and no significant difference was observed between patients and controls or between activated and non-activated cells.

The degree of killing obtained (%KI) was calculated according to the equation:

$$\%\mathrm{KI} = 1 - \left(\frac{A\text{-}B}{X\text{-}Y}\right) \times 100$$

where: A = the mean of triplicate counts of the radioactivity incorporated into monocytes infected with *T. gondii* after stimulation with rγ-IFN or Con A supernatant; B = the mean of triplicate counts of radioactivity incorporated in monocytes treated with rγ-IFN or Con A supernantant alone; X = the mean of triplicate counts of the radioactivity incorporated in monocytes infected with *T. gondii*; Y = the mean of triplicate counts of the radioactivity incorporated in monocytes alone.

Lymphokine production. Two million PBMC obtained as described above were incubated in 1 ml RPMI 1640 (supplemented as before but containing 5% fetal calf serum in place of the human AB serum; RPMI f) in 24-well culture plates. Half the wells were stimulated with Con A (20 μg/ml). After 24 h incubation (37°C; 5% CO_2), 250 μl aliquots were taken from the Con A and control wells and were stored at −20°C until assayed for IL-2 production. Two days later, the remaining supernatants were harvested, centrifuged (600 *g*; 10 min), filtered and stored at −20°C. Con A was added to the control supernatants to compensate for possible residual Con A effects in the stimulated cultures.

Assay for gamma interferon and interleukin 2 production. The gamma interferon (γ-IFN) content of the Con A stimulated supernatants was assayed using a Boots-Celltech immunoradiometric assay (IRMA kit).

Interleukin 2 production was measured using an IL-2 dependent murine cytotoxic T lymphocyte line (CTLL) kindly supplied by Dr B. Champion (Middlesex Hospital, London). Briefly, CTLL cells were washed three times and diluted to 3×10^4 cells/ml in RPMI f containing 10^{-6} M mercaptoethanol. One hundred microlitre aliquots were placed in the wells of a round-bottomed microtitre plate. An equal volume of the test supernatants (or doubling dilutions thereof) was added to the wells. The cultures were incubated for 24 h (37°C; 5% CO_2) with 0·1 μCi/well tritiated thymidine (^{3}H-TdR). Control wells contained cells alone or serial dilutions of a standard recombinant IL-2 (supplied by Biogen, Switzerland). Cultures were harvested and counted as described above.

Statistics. Results were analysed using the Mann-Whitney U test for non-parametric data.

RESULTS

Lymphocyte studies. T cell subsets were enumerated routinely in all patients studied (Table 1). A range of normal values for Leu 3a (CD4), Leu 2a (CD8) and Leu 3a/2a ratios were determined in a pilot study. All values obtained in this report were compared to these normal values.

In the asymptomatic group (AH), 7/28 patients had a statistically significant decrease in the number of Leu 3a cells. The majority of these patients were sero-positive for HTLV-III. By contrast, the majority of AH subjects showing increased Leu 2a numbers (8/14) were HTLV-III −ve. In the PGL group, all patients showed abnormal 3a/2a ratios. In the PGL-A group this was due to a significant increase in the number of Leu 2a cells (6/9 patients) whilst in the PGL-B group the abnormal Leu 3a/2a ratio was largely due to a decrease in the number of Leu 3a cells (7/9 patients). AIDS patients characteristically showed a substantial decrease in Leu 3a numbers leading to reduced Leu 3a/2a ratios.

Con A stimulated IL-2 production. IL-2 production was measured by the incorporation of ^{3}H-TdR in IL-2-dependent cells (CTLL). Supernatants from all groups studied were found to stimulate H-TdR incorporation in the CTLL (Table 2). There was a progressive decline in IL-2 production between groups from AH through PGL to AIDS. This decrease only reached significance in the AIDS group ($P < 0{\cdot}001$). On examination of the sub-groups of AH and PGL, the AH sero-negative group was similar to controls. Unfortunately, for technical reasons only one AH +ve patient was examined in this assay and this patient showed very low levels of IL-2 production.

PGL-A patients surprisingly produced similar levels of IL-2 to controls despite being HIV +ve. By contrast, PGL-B patients produced significantly less IL-2 than controls ($P < 0{\cdot}01$). No correlation was found in any group between IL-2 production and Leu 3a or Leu 2a numbers or Leu 3a/2a ratios.

Production of gamma interferon. The γ-IFN content of Con A stimulated supernatants was measured using a commercially available radiometric assay (Fig. 1). A reduced level of γ-IFN

Table 1. Peripheral blood lymphocyte subpopulations

					% abnormal values	
	n	Th	Ts	H/S	Th	Ts	H/S
NR		>0·53	<0·80	1·20			
Controls	25	0·76 (0·44–1·24)	0·52 (0·26–1·02)	1·57 (0·63–3·65)	12	8	32
AH	28	0·94 (0·08–4·30)	0·80 (0·23–2·10)	1·24 (0·32–3·81)	25	50	57
Sero −ve	14	1·26 (0·40–4·30)	0·84 (0·23–1·80)	1·61 (0·74–3·81)	14	57	36
Sero +ve	14	0·61 (0·08–1·08)	0·76 (0·25–2·10)	0·86 (0·32–1·61)	36	43	79
PGL.	18	0·66 (0·13–1·60)	0·94 (0·19–2·20)	0·69 (0·35–1·15)	44	44	100
Group A	9	0·87 (0·28–1·60)	1·09 (0·33–2·20)	0·82 (0·59–1·15)	11	67	100
Group B	9	0·44 (0·13–1·20)	0·80 (0·19–2·10)	0·57 (0·35–0·77)	78	33	100
AIDS	13	0·24 (0·03–0·78)	0·43 (0·09–1·23)	0·56 (0·28–1·03)	92	15	100

Peripheral blood mononuclear cell subpopulations were evaluated using the monoclonal antibodies Leu 2a (Ts), Leu 3a (Th) and an EPICS V cell analyser. Normal values for Th and Ts cell numbers was determined in a pilot study and all subsequent readings were related to these standards (NR).

Results are shown as the mean (and range in parentheses) and expressed as the number of cells $\times 10^6$/ml.

Table 2. IL-2 production by peripheral blood mononuclear cells

Group	*n*	Spontaneous*	Con A induced
Controls	14	608 (116–1166)	1541 (1061–4634)
AH	8	507 (321–988)	1694 (401–6804)
HIV −ve	7	543 (321–988)	1969 (401–6804)
HIV +ve	1	407	604
PGL	9	501 (235–1131)	1430 (449–2024)
PGL-A	5	603 (235–1131)	1749 (1430–2024)
PGL-B	4	380 (253–501)	883 (449–1213)
AIDS	13	281 (56–900)	503 (79–1836)

PBMC were stimulated with Con A for 24 h after which aliquots of supernatant were assayed for IL-2 production using an IL-2 dependent CTLL.

* Results are expressed as the median (and range in parentheses) of counts per minute from tritiated thymidine incorporated in the CTLL.

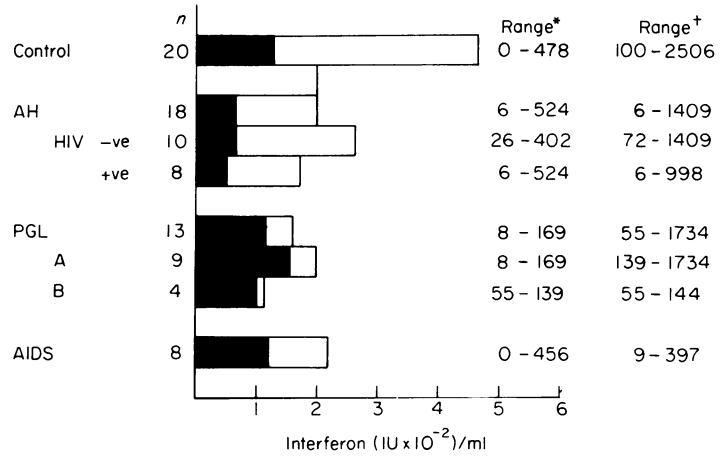

Fig. 1. Gamma interferon production by peripheral blood mononuclear cells. PBMC (2×10^6/ml) were stimulated with Con A for 48 h after which the supernatants were harvested, centrifuged and filtered. Gamma interferon content was assayed using a Boots-Celltech IRMA kit. Bars represent the median number of units ($\times 10^{-2}$)/ml produced either spontaneously (■) or by Con A stimulation (□). Figures represent the ranges for * spontaneous and † Con A induced IFN production (IU $\times 10^{-2}$/ml).

production was seen in all groups examined with respect to controls. This reduction reached significance in both the PGL ($P < 0{\cdot}01$) and AIDS patients ($P < 0{\cdot}05$).

When the AH group was divided according to sero-positivity for HIV it was found that the AH −ve group produced similar levels of γ-IFN to controls whilst the AH +ve group showed a marked reduction ($P < 0{\cdot}05$). Similarly, when the PGL group was subdivided clinically, PGL-B patients showed a significant reduction in γ-IFN production ($P < 0{\cdot}01$) whilst the clinically better PGL-A patients did not. However, no significant difference in γ-IFN production was found between any of the HIV positive groups. No correlation was found between γ-IFN production and IL-2 production or between γ-IFN and T cell subset numbers or ratios.

Killing of T. gondii *by activated monocyte derived macrophages.* In order to study the intracellular killing function of macrophages in AIDS patients and those at risk from AIDS, we examined the ability of monocyte-derived macrophages (hereafter referred to as macrophages) to kill *T. gondii* after stimulation with rγIFN. The results are shown in Fig. 2a. Both AH and PGL groups showed decreased ability to kill *T. gondii* with respect to controls ($P < 0{\cdot}01$). By contrast, AIDS patients showed a similar level of killing to controls. When AH subjects were divided into sero-positive and sero-negative groups, it was found that the latter behaved similarly to control subjects whilst AH +ve patients showed significantly reduced killing of *T. gondii* ($P < 0{\cdot}01$). Interestingly, patients with benign, non-progressive PGL (PGL-A) showed the most profound defect in their ability to kill *T. gondii* ($P < 0{\cdot}01$). By contrast, the killing ability of monocytes from PGL-B patients was similar to that of AIDS patients. No significant difference was found between controls, AH −ve, PGL group B and AIDS patients' macrophages in their ability to kill *T. gondii* after stimulation with γ-IFN. The apparent profound deficiency in the ability of group A PGL patients to kill *T. gondii* was due to the enhanced growth of the organism in the γ-IFN treated cells. This phenomenon occurred in all groups (except AIDS patients) but was much more profound in PGL group A patients (Controls 6%; AH −ve 8%; AH +ve 20%; PGL group A 27%; PGL group B 14%).

When macrophages were incubated with autologous Con A stimulated supernatants, a different pattern was seen. All groups examined showed a statistically similar level of killing despite significant differences in their supernatant γ-IFN content (Fig. 2b). However, if a comparison is made between the killing induced by γ-IFN and that produced by the Con A supernatants, some interesting differences are observed. The control, AH −ve and PGL-B groups showed reduced Con

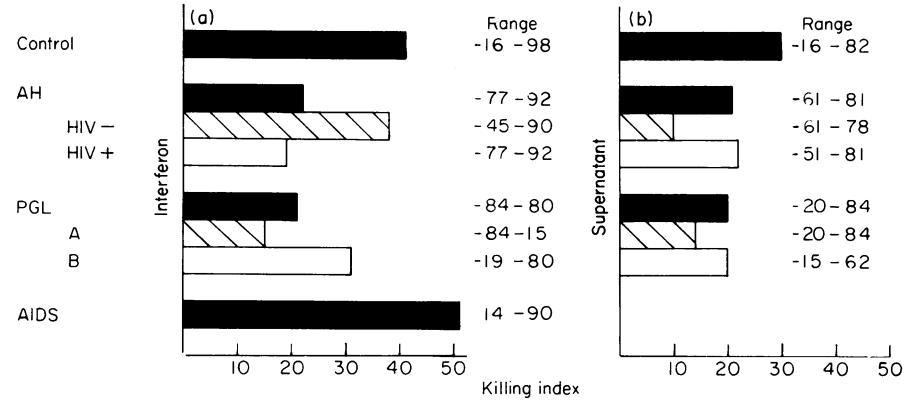

Fig. 2. *Toxoplasma gondii* killing by macrophages activated by (a) rγ-IFN and (b) Con A generated autologous supernatants. (a) Peripheral blood monocytes were cultured for 6 days in RPMI with 30% human AB serum. After stimulation for 24 h with rγ-IFN (250 U/ml) the cells were infected with *T. gondii* for a further 24 h. One microcurie of tritiated uracil was added to each well and the plates incubated for a further 18 h before harvesting. (b) Culture conditions were as described above but cells were stimulated with autologous supernatants (20% v/v) instead of rγ-IFN. Bars represent the group median killing indices and figures represent the ranges. A minus sign before the left hand range figure indicates stimulation of growth of *T. gondii*.

A supernatant induced killing compared with γ-IFN-induced killing. However, this reduction did not reach statistical significance due to the large ranges observed. This result was expected due to the low γ-IFN content of the supernatants. In contrast, the level of killing by macrophages from AH +ve patients stimulated with 250 U rγ-IFN (Fig. 1a) was the same as that produced by stimulation with autologous supernatant containing one thirty-sixth of the concentration of rγ-IFN. This effect was also seen with the PGL patients. PGL-A patients killed as many *Toxoplasma* when stimulated with rα-IFN as when stimulated with autologous supernatant containing thirty-two times less IFN. Also, when killing was considered on a per unit γ-IFN basis, PGL-B patients were 47 times more efficient at responding to natural than rγ-IFN. AIDS patients were not tested in this assay due to the difficulty of obtaining sufficient cell numbers. However, when Con A stimulated supernatants from AIDS patients cells were tested on control macrophages no significant killing was observed (Fig. 3). These results suggest that HIV +ve subjects were able to respond more effectively to non-gamma IFN macrophage activating lymphokines than to rγ-IFN.

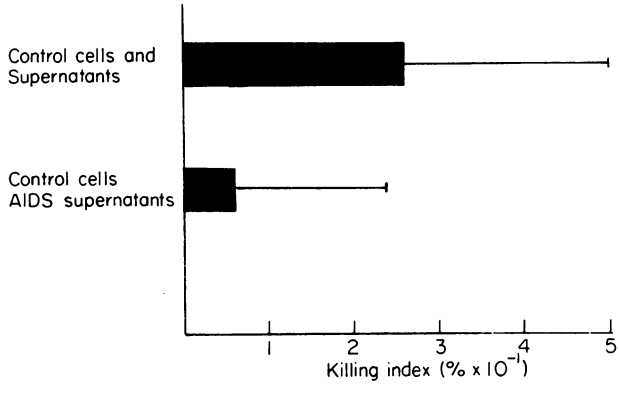

Fig. 3. *Toxoplasma gondii* killing by macrophages from controls activated with autologous or AIDS patients Con A generated supernatants. Bars represent median killing indices and ranges.

DISCUSSION

T cell-macrophage co-operation was studied in patients with AIDS and other HIV related diseases using a radiometric assay. This enabled us to measure not only the ability of patients' lymphocytes to produce macrophage-activating lymphokines but also the capacity of their monocyte derived macrophages to kill intracellular pathogens (Table 3). Similar work has been done previously but using more subjective methods for determining microbicidal activity (Murray *et al.*, 1984; 1985a). The method we have used allows us to quantify the replication of *T. gondii* directly since tritiated uracil incorporation is directly proportional to the number of intracellular organisms (McLeod & Remington, 1979). Furthermore, previous studies have not included the full spectrum of clinical states.

HIV has been shown to infect T cells via the CD4 antigen thus causing selective CD4 lymphopenia (Klatzman *et al.*, 1984a, b; Dalgleish *et al.*, 1984). Functional abnormalities of CD4 cells have also been demonstrated (Lane *et al.*, 1983; 1985; Hofmann *et al.*, 1985; Lin, Tanz & Denny, 1985). These cells are necessary for IL-2 and gamma interferon production and thus these lymphokines may be expected to be deficient in patients infected with HIV (Moretta, 1985; Kasahara *et al.*, 1983; Reem & Yeh, 1984). Indeed, we have confirmed previous reports that cells from patients with AIDS and PGL produce less IL-2 and γ-IFN than control subjects (Alcocer-Varela, Alarcon-Segovia & Abud-Mendoza, 1985; Buimovici-Klein *et al.*, 1984; Murray *et al.*, 1984; 1985b). We have extended their association of this defect with HIV infection by showing that AH +ve subjects also have diminished production of these lymphokines. However, we have not been able to show a direct correlation between the number of $CD4^+$ cells in peripheral blood and the production *in vitro* of either IL-2 or γ-IFN from such cells in any group studied.

Abnormal monocyte function has been demonstrated in patients with AIDS and related diseases (Pinching *et al.*, 1983; Poli *et al.*, 1985). The demonstration of CD4 antigen on monocytes (Wood *et al.*, 1983; Moscicki *et al.*, 1983) and the ability to infect the human monocyte line U937 strongly suggest that these cells may also be infected in AIDS patients. We have confirmed previous observations in that macrophages from patients with AIDS can kill *T. gondii* as efficiently as controls after stimulation with rγ-IFN (Murray *et al.*, 1984; 1985a). We have also shown a similar ability in AH −ve and PGL-B patients. In contrast, HIV +ve individuals were significantly less

Table 3. Summary of the patients' results with respect to controls for each assay studied

Test	AH−	AH+	PGL A	PGL B	AIDS
Leu 3a	0	−	0	−	−−
Leu 2a	+	0	+	0	0
3a/2a	−	−	−	−	−−
IL-2	0	−	0	−	−−
γ-IFN	0	−	−	−	−
Macrophage response to γ-IFN*	0	−−	−	0	0
MAF†	0	0	0	0	N/D
MAF v rγ-IFN‡	0	+	++	++	N/D

0, Equivalent to control response; −, below control response; +, greater than control reponse.

* Macrophage response to gamma interferon.

† Macrophage response to non-gamma interferon activating lymphokines.

‡ Macrophage response to non-gamma activating lymphokines compared with that to gamma interferon.

efficient at killing *T. gondii* in response to stimulation with rγ-IFN. This appeared to be because the rγ-IFN enhanced the growth of *T. gondii*. This phenomenon has been demonstrated previously with other organisms and is dependent upon γ-IFN concentration (Douvas *et al.*, 1985). It is likely that this effect depends on the expression of γ-IFN receptors, a maturation-dependent phenomenon, and thus our results may reflect differences in the maturation of patients' monocytes and hence in their ability to respond to rγ-IFN.

When killing was considered in relation to the number of units of γ-IFN used to stimulate the cultures, we have shown that all HIV +ve subjects (with the exception of AIDS patients) were able to respond more efficiently to natural γ-IFN than to γ-IFN. This may be due to natural γ-IFN being more potent than rγ-IFN or due to the presence of other non-γ-IFN macrophage activating lymphokines. The former explanation seems unlikely since Nathan *et al.* (1983) demonstrated rγ-IFN to be more potent in such an assay than natural γ-IFN. The presence of several non-γ-IFN macrophage activating lymphokines in Con A generated supernatants has previously been reported (Nacy, Leonard & Meltzer, 1981; Andrew *et al.*, 1984; Tzeng *et al.*, 1985) and seems the more likely explanation.

Thus we have shown that the ability of lymphocytes from HIV +ve individuals to produce the lymphokines IL-2 and γ-IFN decreases with progression towards AIDS. Also we have demonstrated indirectly the production of non-γ-IFN macrophage – activating lymphokines. The inability of AIDS patients' supernatants to activate control cells suggests that as the severity of the disease increases, not only the cells responsible for producing γ-IFN, but also those responsible for producing other relevant lymphokines are unable to respond to the stimulus provided by Con A. Finally, the differences we have observed in the response of macrophages to γ-IFN from HIV +ve and −ve individuals may reflect altered receptor expression owing to a difference in monocyte maturation (Hofmann *et al.*, 1984) or subset distribution of the HIV +ve subjects. This difference in maturation may result from activation *in vivo* of patients' monocytes or from direct viral infection. These possibilities are currently being investigated.

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REFERENCES

ALCOCER-VARELA, J., ALARCON-SEGOVIA, D. & ABUD-MENDOZA, C. (1985) Immunoregulatory circuits in the acquired immune deficiency syndrome and related complex. Production of and response to interleukins 1 and 2, NK function and its enhancement by interleukin 2 and kinetics of the autologous mixed lymphocyte reaction. *Clin. exp. Immunol.* **60,** 31.

ANDERSON, S.E. & REMINGTON, J.S. (1974) Effect of normal and activated human macrophages on *Toxoplasma gondii*. *J. exp. Med.* **139,** 1154.

ANDREW, P.W., REES, A.D.M., SCOGING, A., DOBSON, N., MATHEWS, R., WHITTALL, J.T., COATES, A.R.M. & LOWRIE, D.B. (1984) Secretion of a macrophage activating factor distinct from interferon-g by human T cell clones. *Eur. J. Immunol.* **14,** 962.

BARRE-SINOUSSI, F., CHERMANN, J.C., REY, C., NUGEYRE, M.T., CHAMARET, S., GRUEST, J., DAUGUET, C., AZLER-BLIN, C., VEZINET-BRUN, F., ROUZIOUX, C., ROSENBAUM, W. & MONTAGNIER, L. (1983) Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Sciene* **220,** 868.

BUIMOVICI-KLEIN, E., LANGE, M., RAMEY, W.G., GRIECO, M.H. & COOPER, L.Z. (1984) Cell-mediated immune responses in AIDS. *N. Engl. J. Med.* **311,** 328.

DALGLEISH, A.G., BEVERLEY, P.C.L., CLAPHAM, P.R., CRAWFORD, D.H., GREAVES, M.F. & WEISS, R.A. (1984) The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. *Nature* **312,** 763.

DOUVAS, G.S., LOOKER, D.L., VATTER, A.E. & CROWLE, A.J. (1985) Gamma interferon activates human macrophages to become tumoricidal and Leishmanicidal but enhances replication of macrophage-associated mycobacteria. *Infect. Immunity* **50,** 1.

GALLO, R.C., SARIN, P.S., GELMANN, E.P., ROBERT-GUROFF, M., RICHARDSON, E., KALYANAMARAN, V.S., MANN, D., SIDHU, G.D., STAHL, R.E., ZOLLA-PAZNER, S., LEIBOWITCH, J. & POPOVIC, M. (1983) Isolation of human T-cell leukemia virus in acquired immune deficiency syndrome (AIDS). *Science* **220,** 865.

HOFMAN, F.M., LOPEZ, D., HUSMANN, L., MEYER, P.R. & TAYLOR, C.R. (1984) Heterogeneity of macrophage populations in human lymphoid tissue and peripheral blood. *Cell. Immunol.* **88,** 61.

HOFMANN, B., ODUM, N., PLATZ, P., RYDER, L.P., SVEJGAARD, A. & NIELSEN, J.O. (1985) Immunological studies in acquired immunodeficiency syndrome. Functional studies of lymphocyte subpopulations. *Scand. J. Immunol.* **21,** 235.

KASAHARA, T., HOOKS, J.J., DOUGHERTY, S.F. & OPPENHEIM, J.J. (1983) Interleukin 2 mediates immune interferon (IFN-g) production by human T cells and T cell subsets. *J. Immunol.* **130,** 1784.

KLATZMAN, D., BARRE-SINOUSSI, F., NUGEYRE, M.T., DAUGUET, C., VILMER, E., GRISCELLI, C., BRUN-VENZINET, F., ROUZIOUX, C., GLUCKMAN, J-C., CHERMANN, J-C. & MONTAGNIER, L. (1984a) Selective tropism of lymphadenopathy associated virus (LAV) for helper-inducer T lymphocytes. *Science* **225,** 59.

KLATZMAN, D., CHAMPAGNE, E., CHAMARET, S., GRUEST, J., GUETARD, D., HERCEND, T., GLUCKMAN, J-C. & MONTAGNIER, L. (1984b) T-lymphocyte T4 molecule behaves as the receptor for human retrovirus LAV. *Nature* **312,** 767.

LANE, H.C., MASUR, H., EDGAR, L.C., WHALEN, G., ROOK, A.H. & FAUCI, A.S. (1983) Abnormalities of B-cell activation and immunoregulation in patients with the acquired immunodeficiency syndrome. *N. Engl. J. Med.* **309,** 453.

LANE, H.C., DEPPER, J.M., GREENE, W.C., WHALEN, G., WALDMANN, T.A. & FAUCI, A.S. (1985) Qualitative analysis of immune function in patients with the acquired immunodeficiency syndrome. Evidence for a selective defect in soluble antigen recognition. *N. Engl. J. Med.* **313,** 79.

LIN, R.T., TANZ, W.S. & DENNY, T.W. (1985) Suppressor function of T lymphocytes in the acquired immunodeficiency syndrome as assessed by allogeneic mixed lymphocyte culture. *J. Clin. Lab. Immunol.* **16,** 69.

MCLEOD, R. & REMINGTON, J.S. (1979) A method to evaluate the capacity of monocytes and macrophages to inhibit multiplication of an intracellular pathogen. *J. Immunol. Methods* **27,** 19.

MORETTA, A. (1985) Frequency and surface phenotype of human T lymphocytes producing interleukin 2. Analysis by limiting dilution and cell cloning. *Eur. J. Immunol.* **15,** 148.

MOSCICKI, R.A., AMENTO, E.P., KRANE, S.M., KURNICK, J.T. & COLVIN, R.B. (1983) Modulation of surface antigens of a human monocyte cell line, U937, during incubation with T lymphocyte-conditioned medium: detection of T4 antigen and its presence on normal blood monocytes. *J. Immunol.* **131,** 743.

MURRAY, H.W., RUBIN, B.Y., MASUR, H. & ROBERTS, R.B. (1984) Impaired production of lymphokines and immune (gamma) interferon in the acquired immunodeficiency syndrome. *N. Engl. J. Med.* **310,** 883.

MURRAY, H.W., GELLENE, R.A., LIBBY, D.M., ROTHERMEL, C.D. & RUBIN, B.Y. (1985a) Activation of tissue macrophages from AIDS patients: in vitro response of AIDS alveolar macrophages to lymphokines and interferon-g. *J. Immunol.* **135,** 2374.

MURRAY, H.W., HILLMAN, J.K., RUBIN, B.Y., KELLY, C.D., JACOBS, J.L., TYLER, L.W., DONELLY, D.M., CARRIERO, S.M., GODBOLD, J.H. & ROBERTS, R.B. (1985b) Patients at risk for AIDS-related opportunistic infections. Clinical manifestations and impaired gamma interferon production. *N. Engl. J. Med.* **313,** 1504.

NACY, C.A., LEONARD, E.J. & MELTZER, M.S. (1981) Macrophages in resistance to rickettsial infections: characterization of lymphokines that induce rickettsiacidal activity in macrophages. *J. Immunol.* **126,** 204.

NATHAN, C.F., MURRAY, H.W., WIEBE, M.E. & RUBIN, B.Y. (1983) Identification of interferon-g as the lymphokine that activates human macrophage oxidative metabolism and anti-microbial activity. *J. exp. Med.* **158,** 670.

PINCHING, A.J. (1985) Clinical and immunological aspects of the acquired immune deficiency syndrome and related disorders. *J. Hosp. Infect.* **6,** (Supplement C) 1.

PINCHING, A.J., MCMANUS, T.J., JEFFRIES, D.J., MOSHTAEL, O., DONAGHY, M., PARKIN, J.M., MUNDAY, P.E. & HARRIS, J.R.W. (1983) Studies of cellular immunity in male homosexuals in London. *Lancet* **ii,** 126.

PINCHING, A.J. & WEISS, R.A. (1986) AIDS and the spectrum of HTLV-III/LAV infection. *Int. Rev. exp. Pathol.* **28** in press.

POLI, G., BOTTAZZI, B., ACERO, R., BERSANI, L., ROSSI, V., INTRONA, M., LAZZARIN, A., GALLI, M. & MANTOVANI, A. (1985) Monocyte function in intra-venous drug abusers with lymphadenopathy syndrome and in patients with acquired immunodeficiency syndrome: selective impairment of chemotaxis. *Clin. exp. Immunol.* **62,** 136.

REEM, G.H. & YEH, N.H. (1984) Interleukin 2 regulates the expression of its receptor and the synthesis of gamma interferon by human T ells. *Science* **225,** 429.

TZENG, D.Y., DEUEL, T.F., HUANG, J.S. & BAEHNER, R.L. (1985) Platelet derived growth factor promotes human peripheral monocyte activation. *Blood* **66,** 179.

WOOD, GS., WARNER, N.C. & WARNKE, R.A. (1983) Anti-Leu 3/T4 antibodies react with cells of monocyte/macrophage and Langerhans lineage. *J. Immunol.* **131,** 212.