Effect of a non-viral fraction of acquired immunodeficiency syndrome plasma on the vulnerability of lymphocytes to cortisol

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ABSTRACT

We have observed previously that the rate of cortisol catabolism by lymphocytes (CCL) was indicative of the vulnerability of these cells to cortisol. We attempted to ascertain whether cortisol-sensitive lymphocytes (e.g. thymocytes) metabolize cortisol at a different rate from cortisol-resistant cells and whether lymphocytes in which cortisol catabolism is inhibited become cortisol sensitive. The work was facilitated by the observation that an ethanol extract plasma from patients with acquired immunodeficiency syndrome (AIDS) and AIDS-related complex (ARC) had the capacity to inhibit CCL.

The capacity of thymocytes to metabolize cortisol was found to be 11 times lower than that of peripheral lymphocytes. Inhibition of CCL with an ethanol extract of plasma from AIDS/ARC patients made the cells vulnerable to cortisol, causing them to die at a rate seven times greater than that of control samples. It is suggested that these findings may have important implications with regard to the nature of lymphocyte depletion in AIDS/ARC patients or in people at risk of developing the syndrome.

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INTRODUCTION

The ability of lymphocytes to change the molecular structure of cortisol has been demonstrated by a number of workers, including Berliner & Dougherty (1961); Dougherty, Berliner & Berliner (1961); Jenkins & Kemp (1969) and Klein, Kaufmann, Mannheimer & Joshua (1978). Jenkins & Kemp (1969) and Klein *et al.* (1978) demonstrated that human lymphocytes are capable of metabolizing cortisol to tetrahydrocortisol (3α,11β,17α,21-tetrahydroxy-5-pregnan-20-one), 20α-dihydrocortisol and 20β-dihydrocortisol. Klein, Chan & Malkin (1986*b*) found that this pathway of cortisol metabolism is dependent on glucose and can be inhibited by ethanol-extractable compounds found in the plasma of patients with liver disorders and cancer and in that of newborn infants (Klein, Archer & Malkin, 1986*a*). In the present study we noted that plasma from patients with either acquired immunodeficiency syndrome (AIDS) or AIDS-related complex (ARC) also contained ethanol-extractable compounds capable of inhibiting the catabolism of cortisol by lymphocytes (CCL).

Except for a few experiments in which, for example, it was shown that cortisol metabolites have no suppressive effect on lymphocytes (Berliner & Dougherty, 1961; Dougherty *et al.* 1961) or in which a difference in CCL between human T and B cells was demonstrated (Klein, Bessler, Kaufmann *et al.* 1980), no real attempt has been made to establish *in vitro* whether correlation does in fact exist between CCL and their sensitivity to cortisol.

In the present investigation an attempt was made to ascertain first whether there is a correlation between rates of CCL and sensitivity of lymphocytes to cortisol. Rates of CCL and the resistance of lymphocytes to cortisol was measured in three human lymphocyte populations: (1) those resistant to cortisol (peripheral lymphocytes), (2) those in which cortisol catabolism was inhibited by ethanol extracts of AIDS/ARC plasma and (3) those particularly sensitive to cortisol (thymocytes).

We hypothesized that low CCL due either to inhibition or to inherent characteristics of the cells would correlate with the sensitivity of lymphocytes to cortisol.

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MATERIALS AND METHODS

The population of patients comprised eight male homosexuals with reversed T helper/T suppressor (T4/T8) ratios and antibodies positive to human T lymphotrophic retrovirus III (HTLV III). Six had AIDS, four with opportunistic diseases, one with Kaposi's sarcoma and one with pneumocystis carnii pneumonia. The seventh and eighth were diagnosed as having ARC. The patients and healthy donors gave informed consent to the study. Plasma from patients and normal donors was first lyophilized and then extracted with absolute ethanol. The ethanol suspension was centrifuged at 1000 *g* for 10 min and the ethanol supernatant evaporated to dryness. The ethanol-soluble material was taken up in phosphate-buffered saline (PBS) to the original volume of plasma used for the study.

Lymphocytes were isolated from buffy coats of blood bank material obtained within 2 h of the time the blood was donated. Thymocytes were isolated from thymus glands of children undergoing cardiac surgery. The glands were chopped with scissors into tiny pieces in PBS. The slurry was washed with PBS and filtered through gauze. The cells obtained from either buffy coats or thymus glands were centrifuged at 400 *g* using the Ficoll-Isopaque method (Boyum, 1968), washed twice and resuspended in PBS containing 100 i.u. penicillin and streptomycin. Monocytes were separated by adherence to the plastic walls of tissue culture flasks after incubation of the cells at 37 °C in a medium made up of Roswell Park Memorial Institute medium-1640 (RPMI-1640) plus 10% (v/v) fetal calf serum (FCS). RPMI-1640 was made by Gibco Laboratories, New York, NY, U.S.A.; FCS was supplied by Flow Laboratories, McLean, VA, U.S.A. The cells were divided in duplicates into lymphocytes, thymocytes and lymphocytes treated with an ethanol extract of either AIDS/ARC plasma or normal plasma. The capacity of these cell groups to catabolize cortisol and their resistance to cortisol was then measured.

Assay of cortisol catabolism

Each preparation of either lymphocytes or thymocytes was divided into flasks (20 ml glass scintillation vials) so that each flask contained $2{\cdot}5 \times 10^7$ cells in 1 ml suspension (Klein *et al.* 1978). The incubation medium consisted of PBS containing glucose (5·5 mmol/l) which was found to be the most suitable for assessing rates of cortisol catabolism (Klein *et al.* 1986*b*). (Preliminary experiments carried out with lymphocytes immersed in PBS containing glucose with and without plasma and RPMI showed the highest rate of CCL with PBS containing glucose. Viability of the cells was more than 94% after 17 h of incubation in all three media.) Each flask contained 1 ml medium and 1·0 μCi (7×10^5 c.p.m.) cortisol (New England Nuclear, Billerica, MA, U.S.A.; [1,2-^{3}H] cortisol plus non-radioactive cortisol, final concentration 1·4 μmol/l). Viability of the cells (more than 94%) was not altered during incubation with this concentration of cortisol, confirming the findings of Claman (1972).

To examine the effect of ethanol extracts of plasma on CCL, the ethanol extract of plasma of five AIDS/ARC patients and ten controls was made up to an original 0·5 ml plasma.

The sealed flasks were incubated in a shaking bath at 37 °C for 17 h. This time of incubation was chosen after a preliminary experiment in which cortisol catabolism was plotted against time. The reaction was hyperbolic, reaching a plateau after 17 h. The contents of each flask were extracted with chloroform at the end of the incubation period. After evaporating the chloroform, the residues were applied to silica gel HF-254 thin-layer plates (E. Merck, Darmstadt, F.R.G.). The plates were developed in chloroform/methanol (90/100, v/v). All the cortisol metabolites were located in one spot on the plate (Klein *et al.* 1978) and u.v. light (254 nm) was used for detecting the spots of cortisol and its metabolites. The product and substrate spots were scraped off, transferred into scintillation vials and the radioactivity counted. The counts corresponding to the 'metabolites' spot in the blank flask (which contained no cells) were subtracted from the spot count of the metabolites of all samples.

Assay of cortisol sensitivity

The cells were immersed at a concentration of 2×10^6 cells/ml in medium made up of RPMI-1640 plus 10% FCS and incubated at 37 °C in a humidified atmosphere of 5% CO_2 in air. Using the trypan blue exclusion method, cultures were observed daily for cell viability. In one series of experiments, peripheral lymphocytes and thymocytes were immersed in medium containing two different concentrations of cortisol (2·7 and 67 μmol/l). In a second series, lymphocytes were immersed in 1 ml medium with or without cortisol (2·7 μmol/l) and with one of the following additions: (1) a dried ethanol extract of normal plasma equivalent to 1 ml or (2) a dried ethanol extract of plasma from AIDS/ARC patients equivalent to 1 ml.

RESULTS

The viability of lymphocytes and thymocytes incubated for 3 days in the presence or absence of cortisol is shown in Fig. 1. One-way analysis of variance indicated that the overall group effect was significant

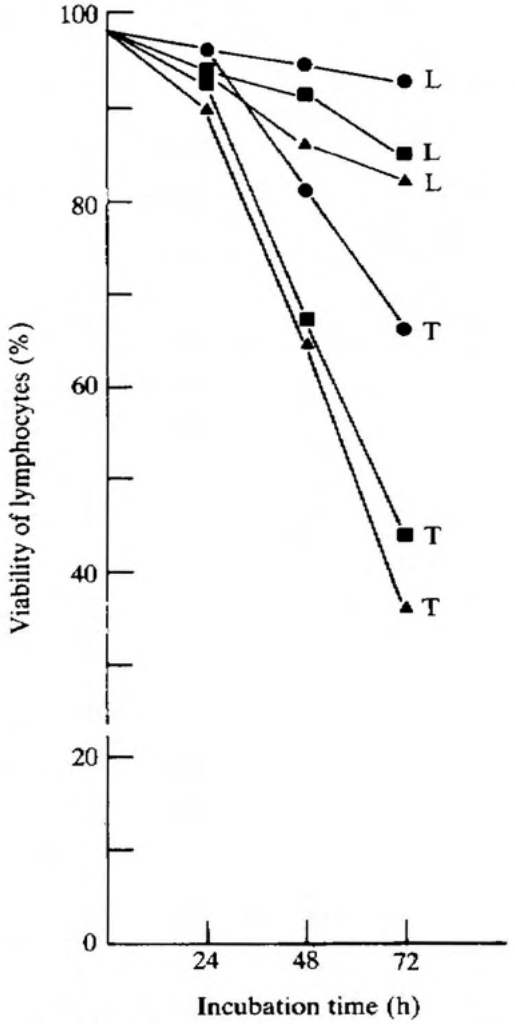

FIGURE 1. Viability of human lymphocytes (L) and thymocytes (T) ($n = 5$) in the absence (●) and presence of 2·7 (■) or 67 (▲) μmol cortisol/l after 3 days of incubation.

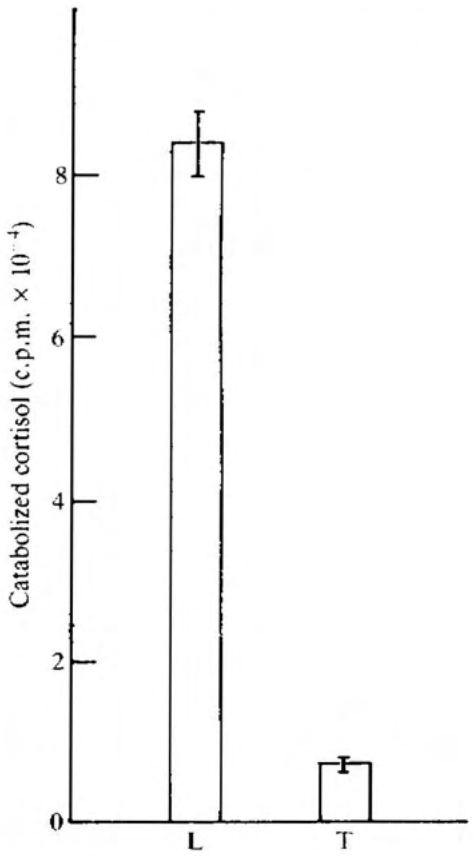

FIGURE 2. Cortisol catabolism by human lymphocytes (L) and thymocytes (T). Experiments were carried out in duplicate and repeated five times with thymocytes and 11 times with lymphocytes. Recovery of radioactivity was more than 87%.

($P < 0{\cdot}0001$). As expected, lymphocytes showed a high resistance to cortisol even at the extreme concentration of 67 μmol/l. (No significant difference between the three lymphocyte groups was detected using Duncan's multiple range test.) A decrease in the viability of thymocytes was noted even in the absence of cortisol ($P < 0{\cdot}05$); however, addition of the steroid augmented this effect ($P < 0{\cdot}05$). Increasing the concentration of cortisol to 67 μmol/l had no significant effect on cell viability as compared with the lower concentration of 2·7 μmol/l.

The difference in the capacity of lymphocytes and thymocytes to catabolize cortisol is illustrated in Fig. 2. The rate obtained with lymphocytes was 11 times greater than that obtained with thymocytes ($P < 0{\cdot}001$, Student's t-test). It should be noted that the viability of the cells after 17 h of incubation was higher than 94%.

The effect of ethanol extracts of 0·5 ml normal plasma and 0·5 ml plasma from AIDS/ARC patients on CCL is illustrated in Fig. 3. Addition of an ethanol extract of normal plasma had no effect on the rate of CCL whereas the addition of an ethanol extract of plasma from AIDS/ARC patients reduced the rate to 38% of that of the control ($P < 0{\cdot}001$, Student's t-test).

The effect on lymphocyte viability of ethanol extracts of either plasma from AIDS/ARC patients or normal plasma in the presence or absence of cortisol is shown in Fig. 4. One-way analysis of variance showed that the overall group effect was significant ($P < 0{\cdot}01$). Duncan's multiple range test showed no significant differences between any of the groups, except for the AIDS/ARC plus cortisol group after both 24 and 48 h of incubation. The difference between the AIDS/ARC plus cortisol group and all other groups was significant after 24 ($P < 0{\cdot}05$) and 48 h ($P < 0{\cdot}01$) of incubation.

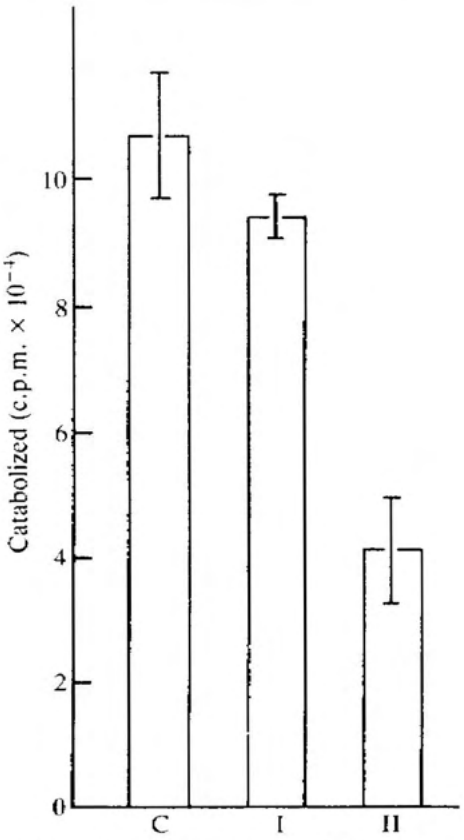

FIGURE 3. Effect of ethanol extracts of 0·5 ml normal plasma (column I) and 0·5 ml plasma from patients with acquired immunodeficiency syndrome (AIDS)/AIDS-related complex (column II) on cortisol catabolism by human lymphocytes. No plasma was added to cells in the control group (C). Values are means ± S.E.M.; *n* (normal subjects) = 10, *n* (patients) = 5, *n* (control) = 10.

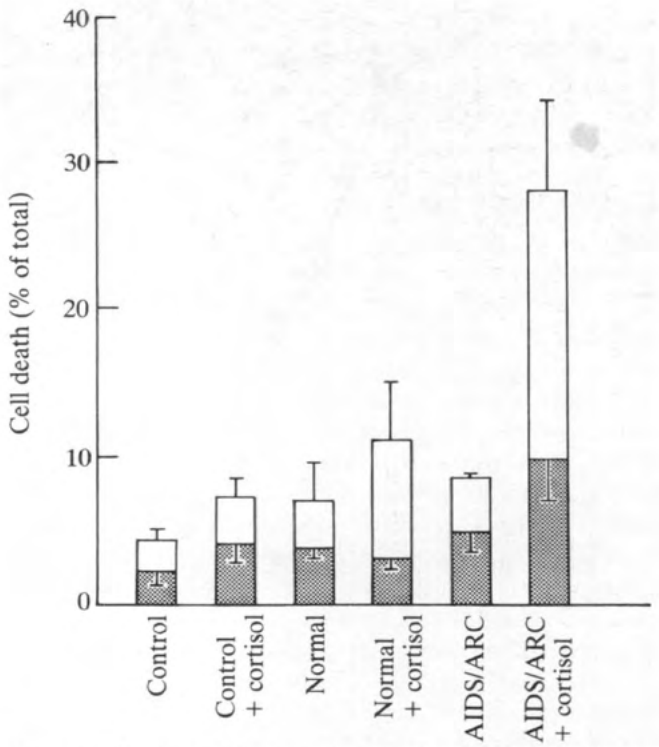

FIGURE 4. Effect on viability of human lymphocytes of ethanol extracts of 1·0 ml plasma from either patients with acquired immunodeficiency syndrome (AIDS) and AIDS-related complex (ARC) or normal subjects in the presence or absence of cortisol (2·7 μmol/l). Cells were incubated for 24 (stippled bars) or 48 h (open bars). Values are means ± S.E.M.; *n* (normal subjects) = 5, *n* (patients) = 4, *n* (control) = 5.

DISCUSSION

Dougherty *et al.* (1961) found that cortisol metabolites have no suppressive effect on lymphocytes. They even postulated that the ability of lymphocytes to metabolize cortisol constitutes an important homeostatic mechanism in the regulation of the lymphocyte population. Noting the in-vivo effect of cortisol on the depletion of peripheral lymphocytes and, in this respect, the greater sensitivity of T cells compared with B cells (Fauci & Dale, 1974; Yu, Clements, Paulus *et al.* 1974; Haynes & Fauci, 1978), Klein *et al.* (1980) found that B cells have a 1·6 times greater capacity than T cells to metabolize cortisol *in vitro*. To our knowledge, except for these preliminary experiments, no attempt has been made to demonstrate whether there is a correlation between CCL and resistance of lymphocytes to cortisol. In the present study an attempt was made to ascertain whether such a correlation exists. The rate of cortisol catabolism by thymocytes, which are cortisol sensitive *in vitro* (Fig. 1) was compared with that of peripheral lymphocytes. After determining that thymocytes have a significantly lower rate of cortisol catabolism than peripheral lymphocytes (Fig. 2) we attempted to establish whether inhibition of CCL might make peripheral lymphocytes vulnerable to cortisol. Since an ethanol extract of plasma from AIDS/ARC patients showed the presence of CCL inhibitors (Fig. 3), it was decided to use this as a means of measuring sensitivity to cortisol of lymphocytes in which cortisol catabolism was either inhibited or uninhibited. After 48 h of incubation in the presence of cortisol plus an ethanol extract of plasma from AIDS/ARC patients, the death rate of lymphocytes rose sevenfold compared with that of the control (Fig. 4). These results provide substantial support for our hypothesis that a correlation exists between the capacity of lymphocytes to catabolize cortisol and their resistance to the steroid.

The fact that only 12% of the added cortisol was metabolized by the lymphocyte preparation (Fig. 2) does not support the hypothesis that this metabolism acts to protect the cells against cortisol. However, the

capacity of the cells to metabolize cortisol depends on the concentration of added cortisol and might rise linearily as much as 500 times (authors' unpublished data). Since activation of intracellular cortisol depends on binding to cortisol receptors, it is possible that the cortisol-metabolizing enzymes are located in a position which allows them to regulate the concentration of receptor-bound cortisol. Furthermore, the hypothesis that the metabolism of cortisol plays a role in protecting lymphocytes against cortisol is supported by our work with the cortisol analogue prednisolone (additional double bond at position 1–2) which is a more potent immunosuppressor and is metabolized much less effectively than cortisol (authors' unpublished data). Thus we still do not know whether inhibition *per se* of the cortisol metabolizing enzymes or their inherent low capability to metabolize the steroid is effective in modulating the sensitivity of lymphocytes to cortisol or is only indicative of other changes.

The isolation and identification of inhibitors of CCL might help us to understand the nature of this phenomenon and its implications with respect to the pathogenesis of AIDS/ARC. We do not as yet have any idea as to the mechanism by which the inhibitors affect CCL. It might occur as a consequence of either a direct effect on the enzymes responsible for cortisol catabolism or, alternatively, an indirect effect on the formation of NADPH and NADH coenzymes necessary for cortisol reduction by lymphocytes (Klein *et al.* 1986*b*). The fact that the cells became sensitive to cortisol after addition of the ethanol extract of plasma from AIDS/ARC patients raises further interesting questions. For example, the presence of a putative inhibitor could result in an intracellular rise in the level of cortisol. On the other hand, the effect on the cell may be such as to make it more vulnerable to proteins produced as a result of cortisol induction. In any event, it appears from the present studies that CCL can be used as a convenient indicator of changes occurring in the cells which make them more vulnerable to the effect of cortisol.

The observations that homosexual males and haemophiliacs are subject to a high incidence of hepatitis (Jones, 1983; McDougal, Jaffe, Cabridilla *et al.* 1985) which, like AIDS/ARC, is characterized by a low T4/T8 ratio (Thomas, 1981; Kern, Rokos & Dietrich, 1984) and that inhibitors of CCL are present in patients with liver disorders due either to disease or immaturity (Klein *et al.* 1986*b*), have led us to investigate the presence of these inhibitors in AIDS/ARC patients. The presence of inhibitors of CCL in the plasma of AIDS/ARC patients suggests the possibility of a link between the high incidence of liver disorders and the frequency of AIDS/ARC among male homosexuals.

It is not clear whether the phenomenon described above is characteristic of those subjects at risk of developing AIDS or whether it is secondary to the damage caused by the virus. However, since (1) the compounds modulating the sensitivity of lymphocytes to cortisol were found in the supernatant fraction of the absolute ethanol extract, (2) lysis occurred earlier than with the HTLV III virus itself (Zagury, Bernard, Leonard *et al.* 1986), i.e. 2 days vs 7–10 days and (3) lysis of lymphocytes occurred only in the presence of cortisol, we surmise that a non-viral component was responsible for the phenomenon.

The nature of the plasma factor(s) responsible for destroying lymphocytes in the presence of cortisol and the possibility that a particular lymphocyte subset is affected is currently under investigation.

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REFERENCES

Berliner, D. L. & Dougherty, T. F. (1961). Hepatic and extrahepatic regulation of corticosteroids. *Pharmacological Reviews* **13**, 329–359.

Boyum, A. (1968). Isolation of mononuclear cells and granulocytes from human blood. *Scandinavian Journal of Laboratory Investigation* **21**, Suppl. 97, 77–89.

Claman, H. N. (1972). Corticosteroids and lymphoid cells. *New England Journal of Medicine* **287**, 388–397.

Dougherty, T. F., Berliner, D. L. & Berliner, M. L. (1961). Corticoid–tissue interactions. *Metabolism* **10**, 966–987.

Fauci, A. S. & Dale, D. C. (1974). The effect of *in vivo* hydrocortisone on subpopulations of human lymphocytes. *Journal of Clinical Investigation* **53**, 240–246.

Haynes, B. F. & Fauci, A. S. (1978). The differential effect of *in vivo* hydrocortisone on the kinetics of subpopulations of human peripheral blood thymus derived lymphocytes. *Journal of Clinical Investigation* **61**, 703–707.

Jenkins, J. S. & Kemp, N. H. (1969). Metabolism of cortisol by human leukemic cells. *Journal of Clinical Endocrinology and Metabolism* **29**, 1217–1221.

Jones, P. (1983). Acquired immunodeficiency syndrome, hepatitis and haemophilia. *British Medical Journal* **287**, 1737–1738.

Kern, P., Rokos, H. & Dietrich, M. (1984). Raised serum neopterin levels and imbalance of T-lymphocyte subsets in viral diseases, acquired immune deficiency and related lymphadenopathy syndrome. *Biomedical Pharmacotherapy* **38**, 407–411.

Klein, A., Archer, S. M. & Malkin, A. (1986*a*). Serum of newborns and patients with cirrhosis or cancer exhibiting inhibitory effect on the metabolism of cortisol by lymphocytes. *Journal of the National Cancer Institute* **77**, 29–31.

Klein, A., Bessler, H., Kaufmann, H., Djaldetti, M. & Joshua, H. (1980). A difference between human B and T lymphocytes. *Journal of Steroid Biochemistry* **13**, 517–520.

Klein, A., Chan, A. W.-L. & Malkin, A. (1986*b*). Effect of glucose, NADH and NADPH on cortisol metabolism by mononuclear cells. *Journal of Endocrinology* **109,** 181–185.

Klein, A., Kaufmann, H., Mannheimer, S. & Joshua, H. (1978). Cortisol metabolism in lymphocytes from cancer-bearing patients. *Metabolism* **27,** 731–736.

McDougal, J. S., Jaffe, H. W., Cabridilla, C. D., Sarngadharan, M. G., Nicholson, J. K. A., Kolyanaraman, V. S., Schable, C. A., Kilbourne, B., Evatt, B. L., Gallo, R. C. & Curran, J. W. (1985). Screening test for blood donors presumed to have transmitted the acquired immunodeficiency syndrome, hepatitis and haemophilia. *Blood* **65,** 772–775.

Thomas, H. C. (1981). T-cell subsets in patients with acute and chronic HBV infection, primary biliary cirrhosis and alcohol induced liver diseases. *International Journal of Immunopharmacology* **3,** 301–305.

Yu, D. T. Y., Clements, P. J., Paulus, H. E. & Peter, J. B. (1974). Human lymphocyte subpopulation, effect of corticosteroid. *Journal of Clinical Investigation* **53,** 565–571.

Zagury, D., Bernard, J., Leonard, R., Cheynier, R., Feldman, M., Sarin, P. S. & Gallow, R. C. (1986). Long-term culture of HTLV-III infected T cells. A model of cytopathology of T cell depletion in AIDS. *Science* **231,** 850–853.