Clin. exp. Immunol. (1989) **78**, 184–188

Impaired autologous mixed lymphocyte reaction (AMLR) reactivity of peripheral blood T cell subsets in rheumatoid arthritis

E. C. KEYSTONE, L. POPLONSKI, K. M. SNOW & M. MARTELL *Rheumatic Disease Unit, Wellesley Hospital and Ontario Cancer Institute, University of Toronto, Toronto, Ontario, Canada*

(Accepted for publication 13 July 1989)

SUMMARY

We examined AMLR reactivity of unseparated T cells and CD4+ and CD8+ T cell subsets in peripheral blood from 11 rheumatoid arthritis (RA) patients and 10 healthy controls. T cell subsets were isolated by negative selection using complement mediated cytotoxicity. AMLR reactivity of six patients (designated RA-L was reduced below the range of the controls' responses. Five patients (designated RA-N) exhibited normal AMLR reactivity. We observed impaired AMLR reactivity of CD4+ T cells from RA-L relative to RA-N and healthy controls ($P < 0.05$). CD4+ T cell reactivity of RA-L was reconstituted to normal with pharmacological doses of recombinant interleukin-2 (IL-2) (100 U/ml). In contrast, CD8+ T cells from RA-L in the presence of 100 U/ml IL-2 exhibited markedly impaired AMLR reactivity relative to RA-N and healthy controls ($P < 0.05$). Dose-response studies revealed partial reconstitution of CD_4 T cells with physiological concentrations of IL-2 (10 U/ml). To examine the possibility that *in vivo* pre-activation of T cells in RA accounted for the findings, T cells or subsets were cultured alone for 7 days in the presence of 100 U/ml IL-2. A trend toward enhanced reactivity of CD4+ and $CD8_+$ T cells in L-RA relative to N-RA and healthy controls was observed, but the differences were not statistically significant. There was no correlation between reactivity of T cells alone in the presence of IL-2 and AMLR reactivity. The results suggest the possibility that abnormal AMLR reactivity of CD4+ and CD8+ T cell subsets in RA may arise as a consequence of different pathophysiological mechanisms.

Keywords autologous mixed lymphocyte reaction T cell reactivity rheumatoid arthritis

INTRODUCTION

Rheumatoid arthritis (RA) is a disorder characterized by cellular and humoral hyper-reactivity locally within the synovium in association with defective peripheral blood (PB) T cell function. One of the most profound and consistent immunological defects of PB T cells reported in RA is impaired responsiveness in the AMLR (Beck *et al.*, 1981; Smith & DeHoratius, 1982; Kalden *et al.*, 1983; Pope *et al.*, 1984).

The AMLR is proliferation that occurs when T cells are co-cultured with autologous non-T cells (Opelz *et al.*, 1975). These T cells require the presence of self-Ia molecules to proliferate (Palacios & Moller, 1981). The AMLR exhibits memory and specificity which are the essential properties of an immune response (Weksler & Kozak, 1977). The nature of the major stimulus in the AMLR, however, is in dispute. Prior exposure of responding T cells to foreign antigens such as sheep red blood cells and fetal calf serum, which are generally used during cell purification or culture, are thought responsible for the observed proliferative response (Huber *et al.*, 1982; Kagen & Choi, 1983). Other data have accumulated, however, indicating that the AMLR is a T cell proliferative response to Ia antigens on stimulator cells (Loffön, Alcocer Varela & Alarcon-Segovia, 1983; Dos Reis & Shevach, 1981). Soluble mediators involved in the AMLR differ, depending on the presence of xenogeneic protein antigens. While interleukin-2 (IL-2) is generated in the AMLR in the presence of xenogeneic protein antigens (Palacios & Moller, 1982; Lattime *et al.*, 1981), a lymphokine with IL-2-like properties, but not IL-2 or interferon-γ, is produced when the AMLR is assayed in the absence of xenogeneic protein antigens (Suzuki *et al.*, 1986).

T cells recovered from an AMLR exhibit immunoregulatory function including helper and suppressor activity (Smith & Knowlton, 1979; Wolos & Smith, 1982). The suppressor activity generated in the AMLR requires the interaction of CD4 bearing suppressor/inducer T cells with class II antigens leading to clonal expansion of CD8+ suppressor T cells through release of interleukins, especially IL-2 (Palacios & Moller, 1981). Consistent with this is the additive proliferative effect of co-culturing

Correspondence: Dr E. C. Keystone, Wellesley Hospital, Ste 655, 160 Wellesley Street East, Toronto M4Y 1J3, Ontario, Canada.

CD4 and CD8$^+$ T cells in the AMLR and the requirement for CD4$^+$ T cells or IL-2 in the AMLR-generated CD8$^+$ T cell proliferative response (Smolen *et al.*, 1981). Within the CD4$^+$ T cell responsive population, CD4$^+$ 2H4$^+$ suppressor/inducer T cells have been implicated as the primary AMLR reactive T cell subset (Romain *et al.*, 1984; Morimoto *et al.*, 1985; Takeuchi *et al.*, 1987). That AMLR reactivity of the CD4$^+$ T cell subset in RA may be impaired is suggested by previous reports of defective release of IL-2 (presumably from CD4$^+$ T cells) in AMLRs from RA patients (Pope *et al.*, 1984). This contrasts with normal suppressor/inducer activity of AMLR-activated CD4$^+$ T cells recently reported in PB T cells from RA patients (Morimoto *et al.*, 1988).

Although AMLR reactivity of unseparated T cells has been well documented in PB of RA patients, there is no information regarding the cellular origin of the T cell defect. Thus, impaired AMLR reactivity in RA may arise from reduced proliferative responses of the CD4$^+$ and/or CD8$^+$ T cell subset. On this account, we examined AMLR induced proliferation of isolated CD4$^+$ and CD8$^+$ T cells from the PB of RA patients and healthy controls. Our results demonstrated impaired AMLR reactivity of both the CD4$^+$ and CD8$^+$ T cell subsets. However, we were able to fully reconstitute AMLR reactivity of CD4 T cells to normal with pharmacological doses of recombinant IL-2, whereas AMLR reactivity of CD8$^+$ T cells remained markedly impaired.

METHODS AND MATERIALS

Patient selection

Eleven women with definite or classical RA seen in the Rheumatic Disease Unit of the Wellesley Hospital were studied. Mean age ($\pm$s.d.) was 46·0$\pm$17·5 years. All patients were receiving a non-steroidal anti-inflammatory drug (NSAID) in combination with a remittive agent (gold, chloroquine, or penicillamine). Patients receiving prednisone or a cytotoxic agent were excluded. Controls consisted of 10 healthy individuals of comparable age selected from hospital personnel.

Cell separation

Peripheral blood mononuclear cells (PBMC) obtained from venous blood were isolated by Ficoll-Hypaque gradient centrifugation (Boyum, 1968). Cells were washed three times with RPMI 1640 (Ontario Cancer Institute; Toronto, Ontario) and resuspended in α medium minus nucleosides (Ontario Cancer Institute) supplemented with 10% fetal calf serum and antibiotics. T cells were isolated by centrifugation of 2-amino ethylisothrouronium-treated sheep red blood cell rosettes over Ficoll-Hypaque gradients (Pellegrino *et al.*, 1975). With this technique, T cell preparations of both RA and controls contained > 90% E-rosette positive (E$^+$) cells. Recovery of T cells in both patient and control preparations exceeded 90% in all cases. Monocyte contamination of the T cell preparation was < 1% in all cases (patients and controls) as determined by non-specific esterase staining.

T cells were separated into subsets of CD8$^+$ bearing and CD4$^+$-bearing cells, using a negative selection technique incorporating two cycles of lysis of the relevant cells with OKT8 and OKT4 monoclonal antibodies, respectively, (Ortho Diagnostics, Toronto, Canada) and complement (Cederlane, Hornby, Canada) as described (Gullberg & Smith, 1986). Purity of the T cell subsets was determined by FACS analysis. In all cases CD4$^+$ T cell preparations contained > 85% CD4$^+$ T cells and < 5% CD8$^+$ T cells, while CD8$^+$ T cell preparations contained > 85% CD8$^+$ T cells and < 5% CD4$^+$ T cells.

AMLR

AMLRs were performed by mixing 100 μl of similar non-T (E$^-$) cells in a concentration of 1×10^6 cells/ml with 100 μl of unseparated T cells or CD4$^+$ or CD8$^+$ T cells in a concentration of 1×10^6 cells/ml in 200 μl Linbro round-bottomed microtitre plates (Flow Laboratories, McLean, VA). The stimulator cells were pre-treated with 2500 rads of gamma radiation using a gamma cell (Ontario Cancer Institute). The cell mixture was set up in triplicate cultures for 6 days in supplemented α-medium after which the cultures were pulsed with 0·1 μCi of ^{3}H-thymidine (^{3}HtdR) (Amersham, Arlington Heights, IL) (18·2 mCi/mM) for 16 h. Thymidine incorporation was measured as ct/min per whole culture using a liquid scintillation system and the data were expressed as geometric mean ct/min.

Statistical analysis

Comparisons between groups were assessed using a Wilcoxon rank sum test where significance was considered at a $P < 0.05$. Spearman's correlation coefficients were obtained where indicated.

RESULTS

Patients selected for study were grouped according to their AMLR response with unseparated T cells. Six patients (designated RA-L) exhibited AMLR reactivity below the range of the control responses. Five patients (designated (RA-N) exhibited AMLR reactivity within the range of the control responses. The median AMLR responses of selected RA-L was markedly reduced (18·1 ct/min $\times 10^3$) compared with the median response of RA-N (66·1 ct/min $\times 10^3$) ($P = 0.008$) and healthy controls (60·3 ct/min $\times 10^3$) ($P = 0.002$) (Fig. 1). Addition of IL-2 in a

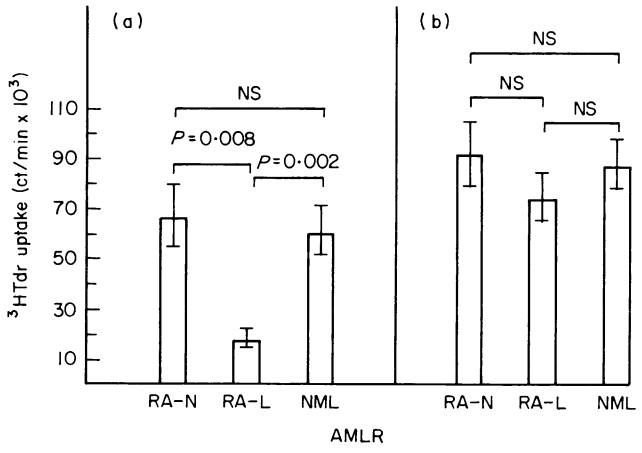

Fig. 1. AMLR reactivity of unseparated responder T cells from patients with rheumatoid arthritis (RA) selected for normal (RA-N) or low (RA-L) AMLR reactivity relative to normal subjects (NML). AMLRs were performed in the absence (a) or presence (b) of 100 U recombinant interleukin-2 (rIL-2). The height of the broad bar represents the geometric mean 3H-thymidine uptake, and error bars represent s.e.m. NS, no significant difference between the groups.

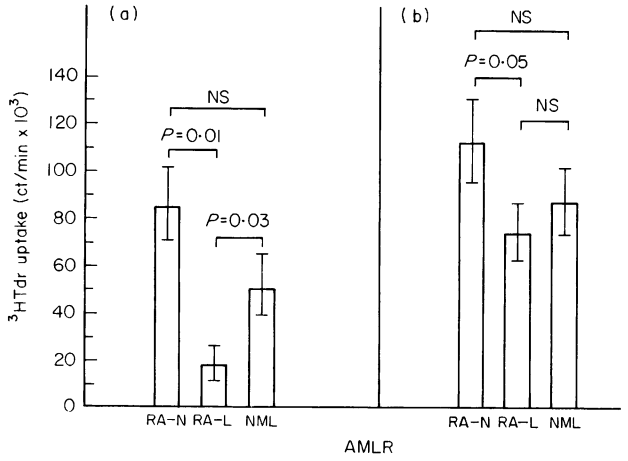

Fig. 2. AMLR reactivity of CD4$^+$ responder T cells from patients with rheumatoid arthritis (RA) selected for normal (RA-N) or low (RA-L) AMLR reactivity relative to normal subjects (NML). AMLRs were performed in the absence (a) or presence (b) of 100 U recombinant interleukin-2 (rIL-2). The height of of the broad bar represents the geometric mean 3H-thymidine uptake, and error bars represent s.e.m. NS, no significant difference between the groups.

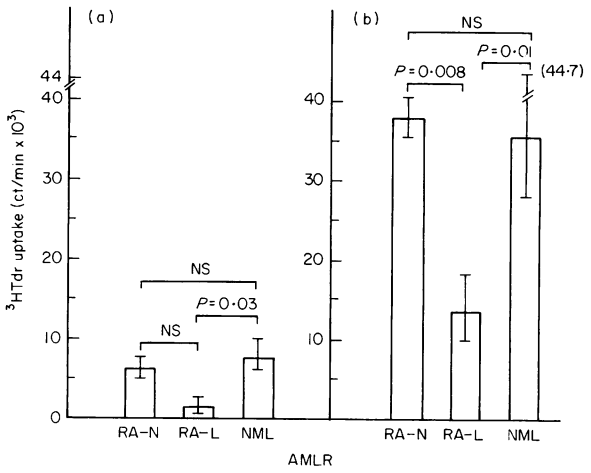

Fig. 3. AMLR reactivity of CD8$^+$ responder T cells from patients with rheumatoid arthritis (RA) selected for normal (RA-N) or low (RA-L) AMLR reactivity relative to normal subjects (NML). AMLRs were performed in the absence (a) or presence (b) of 100 U recombinant interleukin-2 (rIL-2). The height of the broad bar represents the geometric mean 3H-thymidine uptake, and error bars represent s.e.m. NS, no significant difference between the groups.

concentration of 100 U/ml at the initiation of the AMLR cultures resulted in almost complete reconstitution of the response by RA-L (74·0 ct/min x 10^3) to that of RA-N (91·0 ct/min x 10^3) ($P > 0·05$) and healthy controls (87·0 ct/min x 10^3) ($P > 0·05$).

We next attempted to discern whether the low AMLRs in RA-L were a consequence of a reduced response by CD4$^+$ and/or CD8$^+$ T cells. Examination of AMLRs generated with CD4$^+$ responder T cells revealed a marked reduction in the RA-L response (17·8 x 10^3 relative to RA-N (85·0 ct/min x 10^3) ($P = 0·01$) and healthy controls (51·3 ct/min x 10^3) ($P = 0·03$). (Fig. 2a). The median AMLR response of RA-N was enhanced relative to that of healthy controls, although the difference was not statistically significant ($P > 0·05$). As with AMLRs generated with unseparated T cells, addition of 100 U IL-2 to AMLR cultures of CD4$^+$ T cells reconstituted to the level of the RA-L response (74·0 ct/min x 10^3) to that of the healthy controls (87·0 ct/min x 10^3) ($P > 0·05$) (Fig. 2b). IL-2 partially reconstituted the RA-L response to that of RA-N (112.0 ct/min x 10^3) ($P = 0·05$).

AMLR reactivity of isolated CD8$^+$ T cells generated in the absence of IL-2 were low (Fig. 3a). Nevertheless, response of CD8$^+$ T cells from RA-L (1·6 ct/min x 10^3) was reduced relative to that of the median AMLR of RA-N (6·3 ct/min x 10^3) ($P > 0·05$) and healthy controls (7·8 ct/min x 10^3) ($P = 0·03$) (Fig. 3a). In contrast to the effect of IL-2 on AMLR of unseparated and CD4$^+$ responder T cells, IL-2 did not reconstitute the median AMLR response of CD8$^+$ T cells from RA-L (13·5 ct/min x 10^3) to the level of RA-N (38·0 ct/min x 10^3) ($P = 0·008$) or healthy controls (44·0 ct/min x 10^3) ($P = 0·01$) (Fig. 3b).

Reconstitution experiments with IL-2 were performed with pharmacological doses of IL-2. Since the concentration of IL-2 generated in AMLR cultures is in the range of 5–10 U/ml (Pope *et al.*, 1984), we attempted to reconstitute the AMLR responses with a more physiological dose of 10 U/ml of IL-2. The results were similar to those obtained using 100 U/ml of IL-2 (Fig. 4).

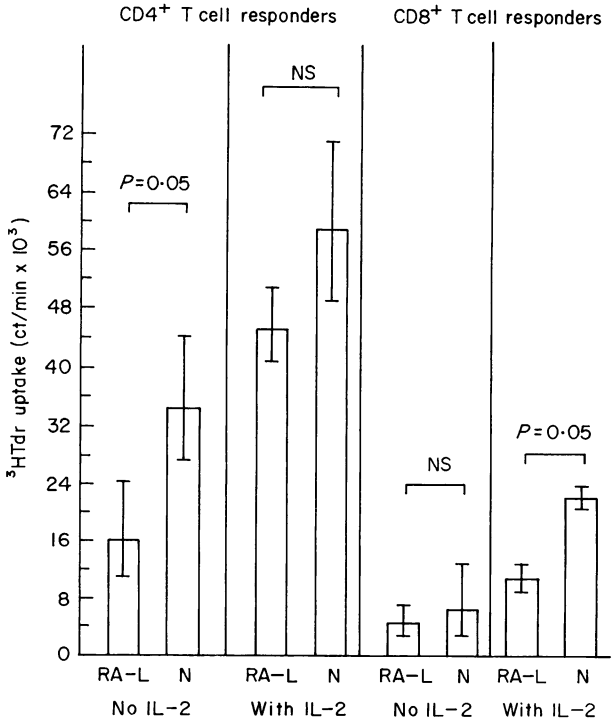

Fig. 4. AMLR reactivity of CD4$^+$ or CD8$^+$ responder T cells from patients with rheumatoid arthritis (RA) selected for normal (RA-N) or low (RA-L) AMLR reactivity relative to normal subjects (NML). AMLRs were performed in the absence or presence of 100 U recombinant interleukin-2 (rIL-2). The height of the broad bar represents the geometric mean 3H-thymidine uptake, and error bars represent s.e.m. NS, no significant difference between the groups.

Table 1. AMLR reactivity of T cells from patients with rheumatoid not receiving remittive drug therapy

	RA		Normal	
	Without IL-2	With 100 U/m IL-2	Without IL-2	With 100 U/ml IL-2
	ct/mm × 10^3		ct/min × 10^3	
Unseparated	28·5	123·2	55·0	73·4
T cells	15·7	40·4	62·5	47·4
	27·2	104·3	52·9	96·7
Log mean	22·9	81·3	56·2	69·3
$CD4^+$ T cells	8·5	155·0	51·8	111·5
	12·7	62·2	55·9	59·6
	21·7	80·3	54·9	98·1
Log mean	13·2	91·2	53·7	87·1
$CD8^+$ T cells	9·8	8·6	4·9	27·0
	1·9	9·4	1·5	40·3
	2·5	7·5	5·0	71·8
Log mean	3·6	8·5	3·3	42·7

3Htdr uptake by T cells from an individual RA patient or healthy age-matched normal.

Thus, 10 U/ml IL-2 added at the initiation of culture almost completely reconstituted the response of $CD4^+$ T cells from RA-L to that of the normals ($P > 0{\cdot}05$) (Fig. 4) while the AMLR response of $CD8^+$ T-cells from RA-L was not reconstituted to that of healthy controls ($P = 0{\cdot}05$) (Fig. 4).

Since the RA patients were receiving at the time of the study drugs that may interfere with our data, we examined three additional patients who were not receiving such therapy within 4 months prior to testing. These patients were receiving NSAID therapy alone. The results (Table 1) were comparable to those in patients receiving remittive therapy. Thus impaired AMLR reactivity of both $CD4^+$ and $CD8^+$ T cell subsets was again observed with reconstitution of AMLR reactivity of $CD4^+$ T cells but not $CD8^+$ T cell with IL-2.

DISCUSSION

The current study confirmed the marked impairment in AMLR reactivity previously demonstrated in the PB of some patients with RA. Our results extend previous observations by demonstrating impaired AMLR reactivity of both the $CD4^+$ and $CD8^+$ T-cell subsets in some RA patients with reconstitution of AMLR reactivity of $CD4^+$ but not $CD8^+$ T cells with IL-2.

IL-2 reconstitution studies suggested that impaired AMLR reactivity in RA may result in part from reduced IL-2 production. This is supported by the demonstration of reduced levels of IL-2 in AMLRs from RA patients (Pope *et al.*, 1984) (unpublished observations).

A reduction in the IL-2 level detected in RA AMLRs could result from the generation of inhibitors of IL-1 or IL-2 which have been described in RA (Lotz *et al.*, 1986; Moissec, Kashiwado & Ziff, 1987). However, we did not find such inhibitors in AMLR-generated supernatants from RA patients (manuscript in preparation). Although impaired AMLR reactivity may result from impaired expresison of IL-2 receptors on AMLR-activated T cells, the reconstitution of AMLR reactivity of unseparated and $CD4^+$ T cells with physiological doses of IL-2 makes this less likely. Impaired expression of IL-2 receptor on $CD8^+$ T cells is possible, however, since IL-2 failed to reconstitute the AMLR response of these cells.

The possibility that drugs impair AMLR reactivity is also a consideration, given previous reports of reduced AMLR reactivity in RA patients receiving remittive therapy (Cross & Hazelton, 1985; Panayi & McKenzie, 1986). Since patients with normal and impaired AMLRs were receiving equivalent NSAIDs and remittive drugs, this explanation is unlikely. Moreover, the results of patients not receiving remittive therapy (Table 1) were comparable to those involving patients receiving these drugs. That treatment of RA patients with NSAIDs accounted for our results, is contradicted by previous data demonstrating enhanced lympocyte function when NSAIDs are administered *in vitro* (Goodwin & Ceuppens, 1983; Goodwin, Bankhurst & Messner, 1977) and *in vivo* (Goodwin *et al.*, 1978) as a consequence of inhibition of prostaglandin E_2 (PGE_2) production. Indeed, in two studies of RA patients NSAIDs significantly enhanced *in vitro* proliferation of lymphocytes (Goodwin, Ceuppens & Rodriguez, 1983; Ceuppens *et al.*, 1986).

The reconstitution of AMLR reactivity of unseparated RA T cells to normal with IL-2 contrasts with a previous report demonstrating little reconstitution of RA AMLRs with IL-2 (Pope *et al.*, 1984). This discrepancy may result from differences in the source of IL-2 utilized. We used recombinant IL-2, while in the previous work a phytohaemagglutinin-induced supernatant provided the IL-2 source. Differences in the timing of IL-2 addition may also account for the discrepant results; IL-2 in our study was added at the initiation of culture, as opposed to the terminal 72 h of culture in the study of Pope *et al.* (1984)

Our results suggest that impaired AMLR reactivity of $CD4^+$ $CD8^+$ T cell subsets arise by different mechanisms. An under-

standing of the mechanisms leading to the differential AMLR reactivity of these T cell subsets may provide further insight into the immunoregulatory defects observed in RA.

ACKNOWLEDGMENTS

We gratefully acknowledge the excellent assistance of Ms Nancy Jellison in the preparation of this manuscript. This study was funded in part by the Arthritis Society of Canada. Recombinant IL-2 was generously supplied by Cetus Corporation.

REFERENCES

BECK, P., BURMESTER, G.R., LEDWOCK, A., URBAN, C. & KALDEN, J.R. (1981) Autologous and allogeneic AMLR-reactivity in patients with rheumatoid arthritis. *J. clin. Lab. Immunol.* **6,** 27.

BOYUM, A. (1968) Isolation of mononuclear cells and granulocytes from human blood. *J. clin. Lab. Invest.* **21** (Suppl 97), 77.

CEUPPENS, J.L., ROBAEYS, G., VERDICKT, W., VERTESSEN, S., DECKMAN, H. & DEQUEKER, J. (1986) Immunomodulatory effects of treatment with Naproxen in patients with rheumatic disease. *Arthritis Rheum.* **29,** 305.

CROSS, S.M. & HAZELTON, R.A. (1985) Correlation of disease activity and drug therapy with the autologous mixed lymphocyte reaction in rheumatoid arthritis. *Ann. rheum. Dis.* **44,** 224.

DOS REIS, G.A. & SHEVACH, E.M. (1981). The mixed leukocyte reaction presents polyclonal activation of antigens-specific T-lymphocytes with receptors for self-Ia antigens. *J. Immunol.* **127,** 2456.

GOODWIN, J. & CEUPPENS, J.L. (1983) Regulation of the immune response by prostaglandin. *J. Clin. Immunol.* **3,** 295.

GOODWIN, J.S., CEUPPENS, J.L. & RODRIGUEZ, M.A. (1983) Effect of non-steroidal anti-inflammatory agents on immunologic function in patients with rheumatoid arthritis. *JAMA*, **250,** 2485.

GOODWIN, J.S., BANKHURST, A.D. & MESSNER, R.P. (1977) Suppression of human T-cell mitogenesis by prostaglandin: existence of a prostaglandin producing cell. *J. exp. Med* **146,** 1719.

GOODWIN, J.S., BANKHURST, A.D., MURPHY, S.A., SELINGER, D.S., MESSNER, R.P. & WILLIAMS, R.C. (1978) Partial reversal of the cellular immune defect in common variable immunodeficiency with indomethacin. *J. clin. lab. immunol.* **1,** 197.

GOTO, M., MIYAMOTO, T., NISHIOKA, K. & UCHIDA, S. (1987) T-cytotoxic and helper cells are markedly increased and T-suppressor & inducer cells are markedly decreased in rheumatoid synovial fluids. *Arthritis Rheum.* **30,** 737.

GULLBERG, M. & SMITH, K.A. (1986) Regulation of T-cell autocrine growth. $T4^+$ cells become refractory to interleukin 2. *J. exp. Med.* **163,** 270.

HUBER, C.M., MERKENSHLAGER, M., GATTRINGER, C., ROYSTON, I., FINK, U. & BRAUNSTEINER, H. (1982) Human autologous mixed lymphocyte reactivity is primarily specific for xenoprotein determinants adsorbed to antigen-presenting cells during rosette formation with sheep erythrocytes. *J. exp. Med.* **155,** 1222.

KAGAN, J. & CHOI, Y.S. (1983) Failure of the human autologous mixed lymphocyte reaction in the absence of foreign antigens. *Eur. J. Immunol.* **13,** 1031.

KALDEN, J.R., LEITNER, O., KLOCKEL, O., KIRCHNER, H., MANGER, B. & KOCH, B. (1983) The autologous mixed lymphocyte reaction in patients with rheumatoid arthritis. *Behring Inst. Mitt.* **72,** 193.

LATTIME, E.C., GILLES, J., DAVID, C. & STUTMAN, O. (1981) Interleukin-2 production in the syngeneic mixed lymphocyte reaction. *Eur. J. Immunol.* **11,** 67.

LOFFÖN, A., ALCOCER-VARELA, J. & ALARCON-SEGOVIA, D. (1983) The autologous mixed lymphocyte reaction is not primary due to xenoantigenic stimulation. *Clin. Immunol. Immunopathol.* **28,** 304.

LOTZ, M., TSOUKAS, C.D., FONG, S., DINARELLO, C., CARSON, D.A., VAUGHAN, J.H. (1986) Release of lymphokines after infection with Epstein–Barr virus *in vitro*. II. A monocyte-dependent inhibitor of interleukin 1 down-regulates the production of interleukin 2 and interferon γ in rheumatoid arthritis. *J. Immunol.* **136,** 3643.

MOISSEC, P., KASHIWADO, T. & ZIFF, M. (1987) Inhibitor of IL-2 in rheumatoid synovial fluid. *Arthritis Rheum.* **30,** 121.

MORIMOTO, C., LETVIN, N.L., DISTASO, J.A., ALDRICH, W.R. & SCHLOSSMAN S.F. (1985) The isolation and characterization of the human helper-inducer T-cell subset. *J. Immunol.* **134,** 3762.

MORIMOTO, C., ROMAIN, P.L., FOX, D.A., ANDERSON, P., DIMAGGIO, M., LEVIN, H. & SCHLOSSMAN, S.S. (1988) Abnormalities in $CD4^+$ T-lymphocyte subsets in inflammatory rheumatic diseases. *Am. J. Med.* **84,** 817.

OPELZ, B., KIUCHI, M., TAKASUGI, M. & TERASAKI, P.I. (1975) Autologous stimulation of human lymphocyte subpopulations. *J. exp. Med.* **142,** 1327.

PALACIOS, R. & MOLLER, G. (1981) HLA-DR antigens reorder resting T-cells sensitive to interleukin-2 and induce production of the growth factor in the autologous mixed lymphocyte reaction. *Cell. Immunol.* **63,** 143.

PANAYI, G.S. & MCKENZIE, M. (1986) Second-line treatment in rheumatoid arthritis associated with depressed autologous mixed lymphocyte reaction. *Rheumatol. Int.* **6,** 25.

PELLEGRINO, M.A., FERRONE, S., DIERICH, P. & REISFIELD, R.A. (1975) Enhancement of sheep red blood cell-human lymphocyte rosette formation by the sulfhydrhyl compound 2-aminoethylisothiouronium bromide. *Clin. Immunol. Immunopathol.* **23,** 324.

PITZALIS, C., KINGSLEY, G., MURPHY, J. & PANAYI, G. (1987) Abnormal distribution of the helper-inducer and suppressor-inducer T-lymphocyte subsets in the rheumatoid joint. *Clin. Immunol. Immunopathol.* **45,** 252.

POPE, R.M., MCCHESNEY, L., TALAL, N. & FISCHBACH, M. (1984) Characterization of the defective autologous mixed lymphocyte response in rheumatoid arthritis. *Arthritis Rheum.* **27,** 1234.

ROMAIN, P.L., MORIMOTO, G., DALEY, J., PALLEY, L.S., REINHERZ, E.L. & SCHLOSSMAN, S.F. (1984) Reactivity of inducer cell subsets and T8 cell activation during the human autologous mixed lymphocyte reaction. *Clin. Immunol. Immunopathol.* **30,** 117.

SMITH, J.B. & DEHORATIUS, R.J. (1982) Deficient autologous mixed lymphocyte reactions correlate with disease activity in systemic lupus erythematosus and rheumatoid arthritis. *Clin. exp. Immunol.* **58,** 155.

SMITH, J.B. & KNOWLES, R.P. (1979) Activation of suppressor T-cells in human autologous mixed lymphocyte culture. *J. Immunol.* **123,** 419.

SMOLEN, J.S., LUGER, T.A., CHUSED, T.M. & STEINBERG, A.D. (1981) Responder cells in the human autologous mixed lymphocyte reaction. *J. clin. Invest.* **68,** 1601.

SUZUKI, R., SUZUKI, S., TAKAHASHI, T. & KUMAGAI, K. (1986) Production and cytokine with interleukin 3-like properties and