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# *In vitro* immunoglobulin synthesis by lymphocytes from patients with rheumatoid arthritis. I. Effect of monocyte depletion and demonstration of an increased proportion of lymphocytes forming rosettes with mouse erythrocytes

CHRISTINE PLATER-ZYBERK, MARY F. CLARKE, KITTY LAM, PAT A. MUMFORD, GERALDINE R.W. ROOM & R.N. MAINI *Division of Clinical Immunology, Kennedy Institute of Rheumatology, London, UK*

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## SUMMARY

We have investigated B cell function in nine patients with rheumatoid arthritis (RA) compared to sex and age matched controls in a pokeweed mitogen driven system. Levels of IgG and IgM synthesized in the supernatant were measured by a competition ELISA. We have found that cultured mononuclear cells from RA patients showed a defective Ig synthesis when depleted of monocytes. In contrast RA mononuclear cells not depleted of monocytes produced substantial levels of Ig after stimulation by the mitogen. The percentages of T and B lymphocytes in the peripheral blood of RA patients were normal; however, an increased number of lymphocytes formed rosettes with mouse erythrocytes indicating an abnormality in the B cell pool. These results demonstrate defective *in vitro* immunoglobulin synthesis by RA lymphocytes and show the importance of monocytes in this culture system.

## INTRODUCTION

Rheumatoid arthritis (RA) is an inflammatory disease of unknown aetiopathogenesis in which immune mechanisms have been considered to play an important role (Yu & Peter, 1974; Zvaifler, 1977). Autoimmune phenomena are seen in the majority of the patients in the form of anti-nuclear antibodies and rheumatoid factors (Venables, Erhardt & Maini, 1980; Vaughan, 1979). The ability of autoantibodies to form complexes and thereby activate complement make them a possible cause of the lesions observed in this disease. The precise mechanism underlying the induction of autoantibodies is unknown. A better understanding of the cellular interactions controlling the synthesis of immunoglobulin in humans should provide insights into the triggering mechanisms of autoantibody production and the means by which this production could be modulated.

The object of this study was to investigate B cell function in RA in a pokeweed mitogen (PWM) driven system in which IgG and IgM production was measured by a sensitive enzyme linked immunosorbent assay (ELISA). We report an impaired production of both IgG and IgM *in vitro* by RA mononuclear cells when depleted of monocytes. This hyporesponsiveness was overcome in the presence of autologous monocytes. The proportions of T and B cells in RA were the same as normals, however an increased number of lymphocytes formed rosettes with mouse erythrocytes.

Correspondence: Christine Plater-Zyberk, Division of Clinical Immunology, The Kennedy Institute of Rheumatology, 6 Bute Gardens, London W6 7DW, UK.

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## MATERIALS AND METHODS

*Study subjects.* Ten patients who fulfilled ARA criteria for RA were studied, each simultaneously with an age and sex matched healthy control. Clinical and serological profiles as well as therapy of the patients are shown on Table 1. None of the patients were taking immunosuppressive drugs. Patients on non-steroid anti-inflammatory drugs were given a wash out period of 18 h.

*Separation of mononuclear cells.* Heparinized peripheral blood, diluted with an equal volume of Hank's balanced salt solution (HBSS) (GIBCO Europe, Paisley, Scotland) was incubated with carbonyl iron (1 mg/ml) for 45 min at 37°C before separation of peripheral blood lymphocytes (PBL) over Ficoll-Hypaque (Histopaque 1077, Sigma, Poole, UK) (Böyum 1968). PBL contained less than 3% residual monocytes. Peripheral blood mononuclear cells (PBM) were separated from blood by an identical procedure but without incubation with carbonyl iron.

Cells were harvested from the Ficoll-Hypaque interface, washed twice in HBSS, and incubated for 1 h at 37°C in RPMI 1640 (Flow Laboratories, Irvine, Scotland) supplemented with 2 mM glutamine, 200 u/ml penicillin and 200 μg/ml streptomycin before being washed twice and resuspended in the same medium containing 10% heat-inactivated fetal calf serum (Flow) (RPMI 10% FCS).

*Cell cultures.* PBL (or PBM) were adjusted to $1{\cdot}25 \times 10^6$ cells/ml in RPMI 10% FCS and aliquots of 200 μl of cells were cultured in flat bottomed microtitre plates (Flow).

PWM (GIBCO) was added as 50 μl aliquots per well at a final concentration of 1·25, 2·25, 5, 10, 20, 40 μg/ml. Controls consisted of equal volumes of RPMI 10% FCS alone added to the appropriate wells. Cultures were incubated for 7 days (unless otherwise stated) in a humified atmosphere of 5% $CO_2$ in air. At the end of the incubation, 165 μl of the supernatant were carefully aspirated and stored at −20°C until assayed. The viability of cells was routinely checked using trypan blue exclusion.

*Identification of mononuclear cells.* T cells were estimated by rosetting with AET Es (Moore & Zuzman, 1978). Cells with stable surface membrane incorporated immunoglobulin (B cells) were estimated by the direct antiglobulin rosette method of Coombs *et al.* (1977). Mouse rosette forming cells (MRFC) were identified by spontaneous rosette formation with fresh mouse erythrocytes, as described by Stathopoulos & Elliot (1974). Toluidine blue was added to rosette preparations before gentle resuspension and a minimum of 250–300 cells were counted by two observers independently

**Table 1.** Clinical and laboratory data of RA patients

| Patient | Sex | Age | IgG (g/l) | IgA (g/l) | IgM (g/l) | C3 (g/l) | C4 (g/l) | ESR (mm/h) | RF (RAHA titre) | Treatment |
|---|---|---|---|---|---|---|---|---|---|---|
| R.G. | M | 33 | 11·3 | 3·0 | 1·0 | 1·3 | 0·33 | 71 | 1/2,560 | N.S.A.I.D.* + Penicillamine |
| C.B. | M | 68 | 15·8 | 4·2 | 3·8 | 1·0 | 0·10 | 30 | 1/10,240 | No treatment |
| I.T. | F | 73 | 10·1 | 2·5 | 2·7 | 1·10 | 0·28 | 66 | Neg | N.S.A.I.D. |
| O.C. | F | 58 | 9·5 | 1·7 | 1·9 | 1·2 | 0·14 | 58 | 1/2,560 | N.S.A.I.D. |
| E.R. | F | 67 | 11 | 2·3 | 1·2 | 1·7 | 0·23 | 25 | 1/5,120 | N.S.A.I.D. |
| D.H. | F | 68 | N.D. | N.D. | N.D. | N.D. | N.D. | 67 | Neg. | N.S.A.I.D. |
| A.P. | M | 69 | 13·1 | 2·7 | 1·2 | 1·7 | 0·22 | 9 | 1/640 | No treatment |
| B.C. | F | 85 | 17·6 | 2·5 | 0·4 | N.D. | N.D. | 51 | 1/160 | No treatment |
| K.C. | F | 67 | 6·0 | 0·8 | 0·8 | 0·9 | 0·14 | 1 | 1/320 | N.S.A.I.D. |
| F.B. | M | 65 | 12·2 | 2·5 | 0·8 | 1·7 | 0·32 | 34 | 1/320 | No treatment |
| Normal values | | | 5·5–14·5 | 0·5–3·2 | 0·5–3·1 | 0·8–1·8 | 0·13–0·43 | < 10 | < 1/40 | |

* N.S.A.I.D. = non-steroidal anti-inflammatory drugs.

with less than 2% variation. Monocytes were estimated by staining of fixed cytocentrifuge cell preparations for α-naphthyl acetate esterase (Sigma kit 90-A1).

*Assay for Ig production.* This was performed by a competition ELISA. Lymphocyte culture supernatants or Ig standards (30 μl) were mixed with 45 μl of alkaline phosphatase conjugated goat anti-human IgG or IgM (Sigma) in round bottomed microtitre plate (Linbro, Flow, UK) which had been coated with 1% BSA in PBS. The antiserum was diluted 1 in 500 with PBS containing 0·1% Tween 20 and 0·5% casein. After incubation at 37°C for 1 h, samples of 60 μl were transferred to an ELISA microtitre plate (Dynatech, Billingshurst, UK) which had been coated with the appropriate Ig at a concentration of 50 μg/ml and then blocked with BSA-PBS. The ELISA plate was then incubated at 37°C for 1 h, washed extensively and finally incubated at 37°C for 30 min with 50 μl of substrate (Alkaline phosphatase Substrate tablets, Sigma; 1 tablet dissolved in 5 ml of glycine buffer, pH 10·4). The optical densities were read at 405 nm on a Titertek Multiscan (Flow). The amounts of Ig in the supernatants were determined using a standard curve of eight dilutions of the reference Ig. This assay is able to measure IgG and IgM in the range of 10–10,000 ng/ml.

*Expression of results and statistical methods.* Immunoglobulin measured in each culture system was corrected for the number of B cells present and expressed as nanograms Ig per $10^5$ B cells. To compare the changes after addition of PWM, stimulation indexes (SI) have been calculated as follows:

$$SI = \frac{(\text{maximum Ig produced with PWM}) - (\text{Ig produced without PWM})}{(\text{Ig produced without PWM})}.$$

Statistical comparisons between groups were performed using Wilcoxon's sum of rank test and *P* values derived from standard tables.

## RESULTS

*Spontaneous and PWM-induced Ig production*

As seen on Fig. 1, normal and RA PBL secreted low levels of IgG after 7 days in culture without exogenous stimulation. PBL from RA patients and healthy matched controls were stimulated by six different concentrations of PWM and the production of IgG assayed at the end of 7 days incubation. The maximal response to PWM is shown in Fig. 1. A marked increment in the amount of IgG synthesized in response to PWM stimulation is a characteristic feature of the majority of normal lymphocyte cultures (SI = 1·5–15·5). In contrast there was a minimal change in IgG production by PBL from RA patients after the addition of the mitogen (SI = 0–1·1). PBL from only one patient (CB) had a normal pattern of IgG production (SI = 3·7). The difference in PWM-induced response between normals and RA PBL was found to be significant ($P < 0·05$). Similarly, significantly less IgM was detected in five RA PBL cultures stimulated by PWM when compared to an equal number of normal controls ($P < 0·02$).

*Effects of monocytes on PWM driven Ig production*

In four experiments, peripheral blood mononuclear cells depleted of monocytes (PBL) and not depleted of monocytes (PBM) were studied in parallel. PBL from RA patients showed hyporesponsiveness to PWM. By contrast stimulation of PBM from the same patient resulted in the production of substantially higher levels of Ig, thus demonstrating the importance of monocytes in this culture system (Fig. 2).

*Enumeration of lymphocytes populations in the peripheral blood*

PBL were assessed for their T and B lymphocytes composition and their ability to form spontaneous rosettes with mouse erythrocytes (MRFC). The latter has been reported to characterize a subset of lymphocytes within the B population (Gupta, Good & Siegal, 1976a; Forbes & Zalewski, 1976). The proportions of T and B lymphocytes were in the normal range, but the MRFC were significantly increased in RA when compared to normal controls, accounting for approximately two-thirds of the B cell population (Table 2). Percentages of monocytes contained in the total PBM populations and PBL preparations were determined by α-naphthyl acetate esterase staining. No significant differences were found between normal and RA preparations (data not shown).

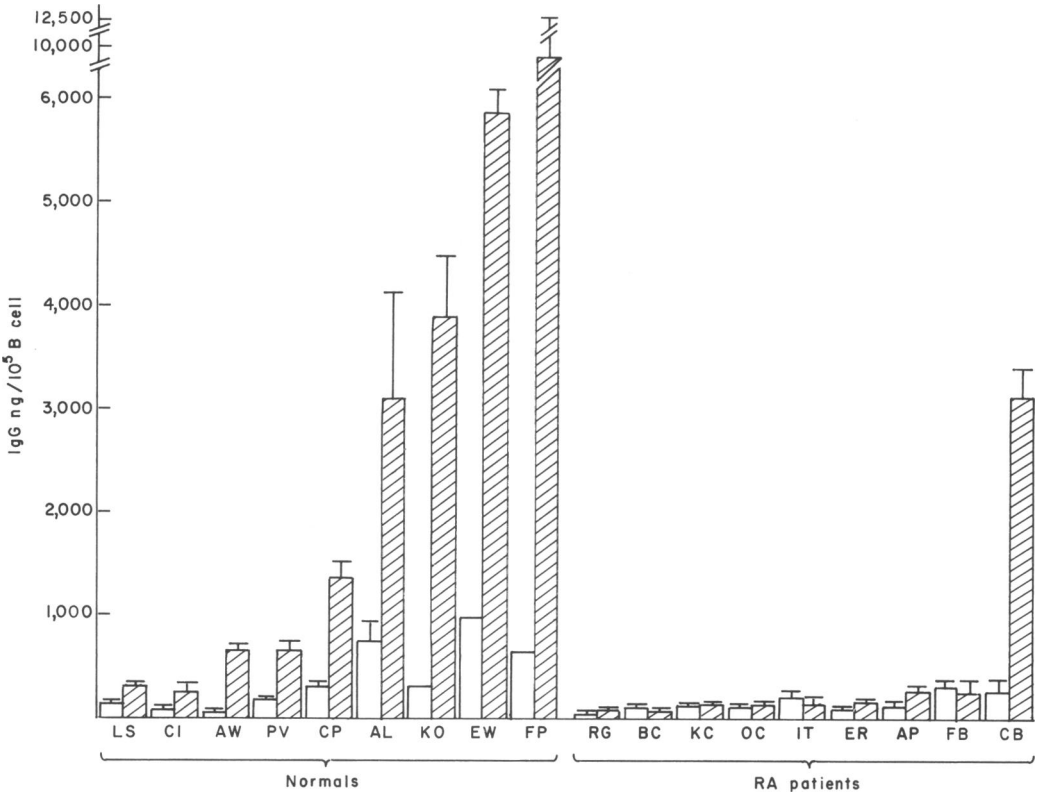


**Fig. 1.** IgG synthesis by normal and RA PBL after 7 days cultures unstimulated (□) and stimulated (▨) by optimal dose of PWM. Results expressed as mean of triplicate cultures, ng/$10^5$ B cells + s.e. (mean).

## DISCUSSION

In this report we describe decreased synthesis of IgG and IgM in response to PWM by PBL of RA patients when compared to normal controls. In contrast, PBM from the same patients are able to produce substantial amounts of Ig in response to the mitogen. Only one of our patients showed a normal response. This may have been due to a failure to fully deplete monocytes resulting in a response characteristic of PBM rather than PBL (Fig. 2).

Other groups, using different culture conditions (e.g. mononuclear cells not depleted of monocytes, macrocultures, longer incubation time) and different assays for Ig detection (radioimmunoassay, plaque forming cell) have reported Ig synthesis by cultured RA cells (Tsoukas *et al.*, 1980; Patel & Panayi, 1982). However depressed levels of IgM in response to PWM (Koopman & Schrohenloher, 1980; Alarcon, Koopman & Schrohenloher, 1982; Olsen, Ziff & Jasin, 1982) and

**Table 2.** Percentage of T, B and MRFC in RA patients and normal controls

| | RA patients ($n=9$)* | Normal controls ($n=9$)* |
|---|---|---|
| T | 67 ± 11 | 72 ± 5 |
| B | 19 ± 2 | 18 ± 2 |
| MRFC | 13 ± 5† | 5 ± 2† |

* % of PBL ± 1 s.d.
† $P < 0{\cdot}001$ (RA *vs* normals).

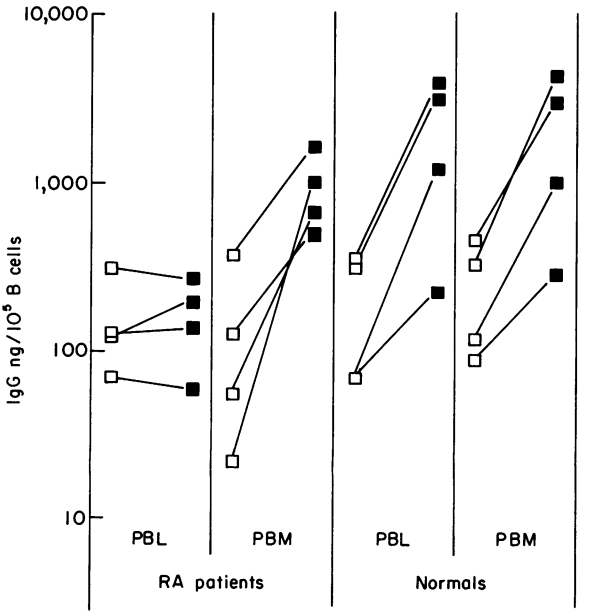


**Fig. 2.** IgG synthesis by normal and RA peripheral blood lymphocytes (PBL) and total mononuclear cells (PBM) after 7 days cultures, unstimulated (□) and stimulated (■) by optimal dose of PWM. Results expressed as mean of triplicate cultures, ng/$10^5$ B cells.

depressed specific primary antibody response to TNP by RA mononuclear cells (Segond *et al.*, 1979) have also been reported.

The hyporesponsiveness observed by us cannot be explained by a decreased survival of RA cells in culture as their viability at the end of the culture period was similar to the normal controls. Cultures have been incubated for up to 11 days without detectable increase in Ig production, thus ruling out differences in the kinetics of the response between patients and controls. An alternative explanation for the low Ig levels may relate to the sensitivity of the assay or the effect of interfering factors. The competition ELISA has been thoroughly evaluated and found to detect as little as 10 ngIg/ml. Our supernatants tested in parallel using the inhibition radioimmunoassay in liquid phase (Pereira, Webster & Platts-Mills, 1982) gave very comparable results to the competition ELISA (data not shown). An interfering factor in the ELISA could be RF secreted in the supernatant by cultured cells. However, we have found that the addition of purified RF does not alter the levels of IgG detected in the assay. Moreover if IgM-RF was produced, normal or high levels of IgM should be detectable. This was not the case. Another possible interference might be cell bound RF which could immobilize secreted IgG on the cell membrane, thus diminishing the levels of IgG in the supernatant. This possibility, although improbable, cannot be ruled out.

Monocytes seem to play an important role in RA cultures since monocyte depleted PBL synthesized substantially lower levels of Ig than total PBM (Fig. 2). In contrast depletion of monocytes from normal PBM did not lead to a diminution of Ig synthesis. Because the proportions of residual monocytes in RA and normal PBL preparations were the same, the results indicate that a greater number of monocytes are required for rheumatoid B cells to respond to PWM. The observed monocyte related effect suggests either a defiency in the RA monocyte in generating sufficient 'helper signals' or a diminished sensitivity of the responding lymphocytes to such signals.

No abnormality in the proportions of T and B lymphocytes in the peripheral blood of RA patients has been detected (Table 2). This is in agreement with other groups (Yu & Peter, 1974; Horwitz & Jull-Nielsen, 1977; Froland & Abrahamsen, 1979). Hyporesponsiveness is therefore not due to quantitative alteration in the proportions of T or B cells present but could still be due to changes in one or more subpopulation(s). T subpopulations have been investigated in RA and there

are conflicting reports on both the proportions and functions of helper and suppressor T cells. Abnormalities of suppressor T cells have been found by some workers (Keystone *et al.*, 1980; Abdou *et al.*, 1981; Duclos *et al.*, 1982; Fox *et al.*, 1982; Raeman *et al.*, 1982; Veys *et al.*, 1982), while others have found contradictory results (Palacios, Ruiz-Arguelles & Alarcon-Segovia, 1981; Meijer *et al.*, 1982; Ranki *et al.*, 1982). In our experiments where variable proportions of T and B lymphocytes were co-cultured, a direct suppression by T lymphocytes could not be demonstrated (data not shown).

Hyporesponsiveness may also be due to abnormality in the B cell population itself. PWM stimulates a very limited number of B cells (Ault & Towle, 1981). This PWM responsive population could be under-represented in the circulation of RA patients. Furthermore, increased numbers of MRFC, a subpopulation of B cells, have been found in the blood of RA patients (Room *et al.*, 1982 and Table 2). MRFC, have been described in chronic lymphocytic leukaemia and in normals as immature B cells (Gupta *et al.*, 1976a, 1976b, 1976c; Forbes *et al.*, 1982). Lucivero, Lawton & Cooper (1981) found that MRFC enriched cultures from normal subjects respond poorly to PWM stimulation. Increased representation of this population in the blood of RA patients could result in low Ig production in our PWM driven cultures. MRFC have also been found to be poor stimulators in autologous and allogeneic MLR (Davey & Kurec, 1982). On the other hand, autologous MLR has been reported as necessary for PWM-induced differentiation of human B lymphocytes (Hee-Sup Shin *et al.*, 1981) and a depressed autologous MLR has been described in a system where RA PBL are used as stimulators (Beck *et al.*, 1981; Kalden *et al.*, 1982).

The above observations lead us to the hypothesis that the increased proportion of MRFC in the peripheral blood of RA patients may be the cause of a depressed autologous MLR. This in turn leads to a depressed differentiation of B lymphocytes resulting in a defective production of Ig. This hyporesponsiveness could be overcome in the presence of monocytes known to be good stimulators of autologous MLR (Hausman & Stobe, 1979; MacDermott & Stacey, 1981). Experiments to differentiate between a defect in the monocyte population itself and/or a defect due to increased numbers of MRFC along with the possible involvement of soluble factors in these interactions are currently under investigation.

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## REFERENCES

ABDOU, N.I., LINDSLEY, H.B., RACELA, L.S., PASCUAL, E. & HASSANEIN, K.M. (1981) Suppressor T cell dysfunction and anti-suppressor cell antibody in active early rheumatoid arthritis. *J. Rheumatol.* **8,** 9.

AULT, K.A. & TOWLE, M. (1981) Human B lymphocytes subsets. I. IgG-bearing B cell response to Pokeweed mitogen. *J. exp. Med.* **153,** 339.

ALARCON, G.S., KOOPMAN, W.J. & SCHROHENLOHER, R.E. (1982) Differential patterns of *in vitro* IgM rheumatoid factor synthesis in seronegative and seropositive rheumatoid arthritis. *Arthrit. Rheum.* **25,** 150.

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

BÖYUM, A. (1968) Isolation of mononuclear cells and granulocytes from human blood: isolation of mononuclear cells by one centrifugation and of granulocytes by combining centrifugation and sedimentation at 1 *g*. *Scand. J. clin. lab. Invest.* Suppl. **21,** 77.

COOMBS, R.R.A., WILSON, A.B., EREMIN, O., GURNER, B.W., HAEGERT, D.G., LAWSON, Y.A., BRIGHT, S. & MUNRO, A.J. (1977) Comparison of the direct antiglobulin rosetting reaction with the mixed antiglobulin rosetting reaction for the detection of immunoglobulin on lymphocytes. *J. Immunol. Meth.* **18,** 45.

DAVEY, F.R. & KUREC, A.S. (1982) Mouse rosette positive B cells stimulate poorly in the autologous and allogeneic mixed lymphocyte reaction. *Clin. exp. Med.* **49,** 193.

DUCLOS, M., PETER, H., ZEIDLER, H., RIEBER, P., PICHLER, J. & LIMAN, W. (1982) Lymphocyte subpopulations determined with monoclonal antibodies (OKT series) in synovial fluid and peripheral blood of patients with rheumatoid arthritis and other joint diseases. *Clin. Rheum.* **1,** 51.

FORBES, I.J. & ZALEWSKI, P.D. (1976) A subpopulation of human B lymphocytes that rosette with mouse erythrocytes. *Clin. exp. Immunol.* **26,** 90.

FORBES, I.G., ZALEWSKI, P.D., VALENTE, L. & GEE, D. (1982) Two maturation-associated mouse erythrocyte receptors of human B cells. I. Identification of four human B-cell subsets. *Clin. exp. Immunol.* **47,** 396.

FROLAND, S.S. & ABRAHAMSEN, T.G. (1979) Lymphocyte populations in blood synovial fluid and synovial tissue in rheumatoid arthritis. In *Immunopathogenesis of Rheumatoid Arthritis* (ed. by G.S. Panayi & P.M. Johnson) pp. 25. Reedbooks, Chertsey.

FOX, R.I., FONG, S., SABHARWAL, N., CARSTENS, S.A., KUNG, P.C. & VAUGHAN, J.H. (1982) Synovial fluid lymphocytes differ from peripheral blood lymphocytes in patients with rheumatoid arthritis. *J. Immunol.* **128,** 351.

GUPTA, S., GOOD, R.A. & SIEGAL, F.P. (1976a) Rosette formation with mouse erythrocytes. II. A marker for human B and non-T lymphocytes. *Clin. exp. Immunol.* **25,** 319.

GUPTA, S., GOOD, R.A. & SIEGAL, F.P. (1976b) Rosette formation with mouse erythrocytes. III. Studies in patients with primary immunodeficiency and lymphoproliferative disorders. *Clin. exp. Immunol.* **26,** 204.

GUPTA, S., PAWA, R., O'REILLY, R., GOOD, R.A. & SIEGAL, F.P. (1976c) Ontogeny of lymphocyte subpopulations in human fetal liver. *Proc. Natl. Acad. Sci. USA.* **73,** 919.

HAUSMAN, P.B. & STOBO, J.D. (1979) Specificity and function of human autologous reactive T cell. *J. exp. Med.* **149,** 1537.

HEE-SUP SHIN, CHANG-YI, W. & YONG SUNG, C. (1981) Activation of autologous reactive helper T lymphocytes for differentiation of human B lymphocytes. *J. Immunol.* **126,** 2485.

HORWITZ, D.A. & JULL-NIELSEN, K. (1977) Human blood L lymphocytes in patients with active systemic lupus erythematosus, rheumatoid arthritis and scleroderma; a comparison with T and B cells. *Clin. exp. Immunol.* **30,** 370.

KALDEN, R., LEITNER, O., MANGER, A., BECK, A. & KOCH, B. (1982) Impaired autologous mixed lymphocyte reactions between T and non T cells in patients with rheumatoid arthritis. *Clin. Rheum.* **1,** 58.

KEYSTONE, E.C., GLADMAN, D.D., BUCHANAN, R., CANE, D. & POPLONSKI, L. (1980) Impaired antigen-specific suppressor cell activity in patients with rheumatoid arthritis. *Arthrit. Rheum.* **23,** 1246.

KOOPMAN, W.J. & SCHROHENLOHER, R.E. (1980) Enhanced *in vitro* synthesis of IgM rheumatoid factor in rheumatoid arthritis. *Arthrit. Rheum.* **23,** 985.

LUCIVERO, G., LAWTON, A.R. & COOPER, D.M. (1981) Rosette formation with mouse erythrocytes defines a population of human B lymphocytes unresponsive to pokeweed mitogen. *Clin. exp. Immunol.* **45,** 185.

MACDERMOTT, R.P. & STACEY, M.C. (1981) Further characterization of the human autologous mixed leukocytes reaction (MLR). *J. Immunol.* **126,** 729.

MEIJER, C.J.L., LAFEBER, G.J., CNOSSEN, J., DAMSTEEG, M.G. & CATS, A. (1982) T lymphocyte subpopulation in rheumatoid arthritis. *J. Rheumatol.* **9,** 18.

MOORE, A.L. & ZUZMAN, J. (1978) Conditions enhancing the percentage and size of E rosettes formed by T lymphocytes. *J. Immunol. Meth.* **23,** 275.

OLSEN, N., ZIFF, M. & JASIN, H.E. (1982) *In vitro* synthesis of immunoglobulins and IgM-rheumatoid factor by blood mononuclear cells of patients with rheumatoid arthritis. *Rheum. Internat.* **2,** 59.

PALACIOS, R., RUIZ-ARGUELLES, A. & ALARCON-SEGOVIA, D. (1981) Human post-thymic precursor cells in healthy and disease. IX. Immunoregulatory T cell circuits in peripheral blood of patients with rheumatoid arthritis. *Clin. exp. Immunol.* **43,** 473.

PATEL, V. & PANAYI, G.S. (1982) *In vitro* immunoglobulin synthesis by peripheral blood lymphocytes in rheumatoid arthritis. *Clin. Rheum.* **1,** 66.

PEREIRA, S., WEBSTER, D. & PLATTS-MILLS, T. (1982) Immature B cells in fetal development and immunodeficiency; studies of IgM, IgG, IgA and IgD production *in vitro* using Epstein–Barr virus activation. *Eur. J. Immunol.* **12,** 540.

RAEMAN, F., DE COCK, W., LEEMPOELS, J., DE CREE, J. & VERHAEGEN, H. (1982) Enumeration of T-lymphocytes and T-lymphocyte subsets in rheumatoid arthritis using monoclonal antibodies. *Clin. Rheum.* **1,** 67.

RANKI, A., PAAVONEN, T., TOLVANEN, E., KONTTINEN, Y., KANKAANPAA, U., NILSSON, E., BIBERFELD, G. & REITAMO, S. (1982) A discrepancy between T cell phenotype and function in rheumatoid arthritis? *Clin. Rheum.* **1,** 68.

ROOM, G.R.W., PLATER-ZYBERK, C., CLARKE, M.F. & MAINI, R.N. (1982) B lymphocyte subpopulation which forms rosettes with mouse erythrocytes increased in rheumatoid arthritis. *Rheumatol. Int.* **2,** 175.

SEGOND, P., DELFRAISSY, J.F., GALANAUD, P., WALLON, C., MASSIAS, P. & DORMONT, J. (1979) Depressed primary *in vitro* antibody response in rheumatoid arthritis. *Clin. exp. Immunol.* **37,** 196.

STATHOPOULOS, G. & ELLIOT, E.V. (1974) Formation of mouse or sheep red-blood-cell rosettes by lymphocytes from normal and leukemic individuals. *Lancet*, **i,** 600.

TSOUKAS, C.D., CARSON, D.A., FONG, S., PASQUALI, J.L. & VAUGHAN, J.H. (1980) Cellular requirements for pokeweed-mitogen-induced autoantibody production in rheumatoid arthritis. *J. Immunol.* **125,** 1125.

VAUGHAN, J.H. (1979) Rheumatoid arthritis, rheumatoid factor and the Epstein–Barr virus. *J. Rheumatol.* **6,** 381.

VENABLES, P.J.W., ERHARDT, C.C. & MAINI, R.N. (1980) Antibodies to extractable nuclear antigens in rheumatoid arthritis: relationship to vasculitis and circulating immune complexes. *Clin. exp. Immunol.* **39,** 146.

VEYS, E.M., HERMANS, P., SCHNIDLER, J., KUNG, P.C., GOLDSTEIN, G., SYMOENS, S. & VAN WAUVE, J. (1982) Evaluation of T cell subsets with monoclonal antibodies in patient with rheumatoid arthritis. *J. Rheumatol.* **9,** 25.

YU, D.T. & PETER, J.B. (1974) Cellular immunological aspects of rheumatoid arthritis. *Sem. Arthrit. Rheumatol.* **4,** 25.

ZVAIFLER, N.J. (1977) Rheumatoid arthritis. In *Autoimmunity* (ed. by N. Talal). Chap. 19. Academic Press, New York.