Ann. rheum. Dis. (1974), 33, 248

Humoral immunity in juvenile rheumatoid arthritis

HANS M. HÖYERAAL AND OVE J. MELLBYE
Institute of Immunology and Rheumatology, Rikshospitalet and Oslo Sanitetsforening University Hospitals, Oslo 1, Norway

Autoantibodies are not infrequently found in sera from patients with juvenile rheumatoid arthritis (JRA), and recent studies suggest that humoral immune responses are responsible, in part, for the inflammatory synovitis observed in these patients. Whether these antibodies result from non-specific antigenic stimulation of an abnormal immunological apparatus, or from specific antigenic stimulation in a normal immunological apparatus, is not known. We know of no controlled study of the antibody response after antigen stimulation in patients with JRA.

Reports from such studies in adult patients with rheumatoid arthritis (RA) have given conflicting results. Meiselas, Zingale, Lee, Richman, and Siegel (1961) found increased responses to *Brucella* immunization, whereas Shearn, Epstein, and Engleman (1963) and Waller, Ellman, and Toone (1966) found normal responses, and Bandilla, Pitts, and McDuffie (1970) found a selective deficiency in IgM antibody production. Similar conflicting results have been reported in the response to tetanus toxoid. Greenwood and Barr (1960) showed higher responses than in the controls, and Vaughan and Butler (1962) found no difference in the response, whereas Barr, Buchanan, Doniach, and Roitt (1964), and Whaley, Glen, MacSween, Deodhar, Dick, Nuki, Williamson, and Buchanan (1971) found lower responses. Decreased responses in patients with RA have also been observed after immunizations with incompatible blood group antigens A and B (Creger, Choy, and Rantz, 1951; Rawson, Abelson, and McCarty, 1961). Recently, Hazleman and Currey (1973) reported normal antibody responses after primary monovalent typhoid immunization in seropositive patients with RA. These conflicting results could be due to the different immunization procedures used, individual variations in the responses to various antigens, and the use of inadequate controls.

The purpose of the present investigation was to study the immunological apparatus in patients with JRA. This presentation describes the results obtained by testing for antibody production after immunization with microbial antigens, in a group of patients and in a matched group of healthy controls. In addition, comparisons of the serum levels of immunoglobulins and naturally-occurring antibodies were performed. A report of delayed hypersensitivity in the same individuals is given elsewhere (Höyeraal, 1973).

Materials and methods

PATIENTS AND CONTROLS

34 hospitalized patients with JRA, diagnosed according to the criteria of Ansell and Bywaters (1969), and 34 non-hospitalized healthy controls matched in age and sex were studied. There were 22 female and 12 male pairs with a mean age of 9·1 years (2 range to 16). Examination for rheumatoid factor was performed by a modified Waaler-Rose test, latex slide test (Hyland, Los Angeles, Calif., USA), and Ripley test, and antibody to pepsin site of IgG was titrated as described by Mellbye and Natvig (1970). Antinuclear antibodies were tested using undiluted sera by indirect immunofluorescence on acetone-fixed (10 min. at 4°C.) frozen sections from mouse liver (Munthe, 1970) and by an indirect LE test (Larsen and Böyum, 1972). Titration of immunoconglutinin was performed as described by Coombs, Coombs, and Ingram (1961). The frequency of these autoantibodies in the two groups are summarized in Table I. Further clinical and laboratory details of the patients and the controls have been given in a previous report (Höyeraal, 1973).

COLLECTION AND STORAGE OF SERA

Serum was separated within a few hours and stored at −20°C. until analysed. Sera from each patient/control pair were always tested together.

ANTIBODY PRODUCTION AFTER IMMUNIZATION

The antigen used for testing of the primary humoral response was *Brucella*, since the typhoid paratyphoid A and B vaccine first tried, gave unpleasant side-effects. For testing the secondary response, diphtheria and tetanus vaccines were used (Fudenberg, Good, Goodman, Hitzig, Kunkel, Roitt, Rosen, Rowe, Seligmann, and Soothill, 1971). Blood was taken before and 2 weeks after subcutaneous injection of the vaccines. The vaccines and

Accepted for publication September 12, 1973.

Table I *Frequency of autoantibodies in 34 patients with JRA and in 34 age- and sex-matched healthy controls*

Autoantibodies	*Serum dilution*	*JRA*		*Controls*	
		No.	*Per cent.*	*No.*	*Per cent.*
Rheumatoid factors					
Waaler	1/64	3	9	0	0
Latex	1/16	7	21	1	3
Ripley	1/16	5	15	0	0
Pepsin agglutinator	1/8	17	50	15	44
ANF	1/1	12	35	0	0
LE	1/1	0	0	—*	
Immunoconglutinin	1/2	21	62	12	35

*=not tested.

doses used were: 0·5 ml. Brucellin antigen (made from *B. abortus*, *B. melitensis*, and *B. suis*, 2805M from Merck, Sharp, and Dohme, West Point, Pa., USA) or 0·2 ml. *Brucella abortus* Bang vaccine (5-IV-69, WHO International Laboratory for Biological Standards, Copenhagen, Denmark) containing 10^9 bacteria/ml. and 0·5 ml. of the combined diphtheria and tetanus vaccine (9371, National Institute of Public Health, Oslo, Norway) containing at least five flocculation units of diphtheria and one international unit of tetanus. The same type and batch of vaccine was given to each pair.

Titration of antibodies to *Brucella* was performed by a modification of the tube agglutination method described by Hall and Manion (1953). V-disposable microtitre plates were used. 25 µl. serum was mixed with 25 µl. normal saline and 25 µl. Bacto-*Brucella abortus* Antigen (553205, DIFCO, Detroit, Michigan, USA) diluted 1:20 in normal saline. To test for IgG antibodies, 0·2 M 2-mercapto-ethanol (ME) (Type 1, Sigma Chemical Company, St. Louis, Missouri, USA) was added instead of normal saline. The plates remained at room temperature for 1 hr. after addition of normal saline or ME and at 37°C. overnight after addition of antigen. The titre was read with the plates at an angle of 60° after centrifugation at 35 g. for 5 min. Bacto-*Brucella abortus* Antiserum (556510, DIFCO, Detroit, Michigan, USA) was used as positive control.

Density gradient ultracentrifugation was performed in a Spinco preparative ultracentrifuge type L50, using the technique of Fudenberg and Kunkel (1957). The sucrose solutions, 10 to 40 per cent., were prepared in phosphate buffered saline (PBS) (1 volume of 0·1 M phosphate buffer at pH 7·2 to 9 volumes of saline). The fractions obtained were dialysed after centrifugation. Total protein concentration was measured by the Folin method, using a modification by Lowry, Rosebrough, Farr, and Randall (1951). Subsequent titrations of the fractions were performed as described above.

For quantitation of diphtheria and tetanus antibodies, a reversed modification of the electroimmunoassay, or rocket immunoelectrophoresis, of Laurell was used (Höyeraal, Vandvik, and Mellbye, to be published). 10 µl. serum samples were applied in 3 mm. wells in agarose gel near the anode. The agarose was mixed with purified diphtheria or tetanus toxoid and a citrate phosphate buffer with pH 5·2, and the electrophoresis was run at 4 volts/cm. for 3 hrs. The toxoids were obtained from Dr. Nagel, National Institute of Public Health, Bilthoven, The Netherlands. Reference antisera were obtained from the WHO International Laboratory for Biological Standards, Copenhagen, Denmark, and from the National Institute of Public Health, Oslo, Norway.

IMMUNOGLOBULINS

Quantitation of IgG, IgA, and IgM was performed by radial immuno-diffusion in gel technique (Mancini, Carbonara, and Heremans, 1965). For IgG quantitation a 1 per cent. mixture of A 37 and A 45 agarose (L'Industrie Biologique Francaise S.A., Gennevilliers, France) and absorbed anti-IgG obtained by immunization of rabbit 216 with IgG (Kabi, Stockholm, Sweden) was used. For IgA and IgM quantitation, tripartigen plates (Behringwerke, Marburg, Germany) were used.

NATURALLY-OCCURRING ANTIBODIES

Titration of iso-haemagglutinins, anti-A and anti-B, was performed by mixing 1 drop of doubling dilutions of inactivated (30 min. at 56°C.) serum, 1 drop of PBS, and 1 per cent. A_1 or B cells in tubes. These were left at room temperature for 15 min., and centrifuged for 1 min. in an Adams Sero-fuge. The agglutination was read by gently shaking the tube.

Titration of naturally-occurring antibodies to rabbit erythrocytes was performed as for iso-haemagglutinins, except that rabbit erythrocytes were used instead of human erythrocytes.

STATISTICS

The Wilcoxon test for pair differences (Diem, 1962), two-sided, was used when comparing the results obtained in the patient and control groups. A non-parametric test was chosen because some of the factors measured were not normally distributed. When comparing values in two groups of non-paired individuals, *e.g.* rheumatoid factor positive and negative patients, a two-sided Wilcoxon test for two samples was used.

Results

PRIMARY IMMUNE RESPONSE

Before immunization both patients with JRA and their controls had very low levels of antibodies to *Brucella* (Fig. 1, overleaf). Both groups had a significant antibody response after vaccination, but no significant ($P > 0·05$) difference was found between the two groups. Titrations after ME-treatment of the sera showed that there was little IgG antibody, conforming with a primary response. This was confirmed by titration of the fractions obtained by density gradient ultracentrifugation of four sera, which proved to have virtually only antibodies of the IgM type. In contrast, two sera from individuals,

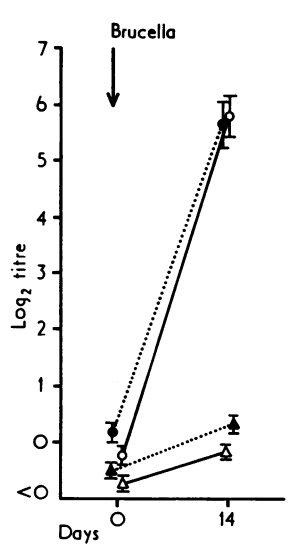

FIG. 1 Brucella *agglutinin titres before and after immunization (↓) in 34 patients with JRA (closed symbols and dotted lines) and their matched healthy controls (open symbols and continuous lines). Circles indicate titres in untreated sera and triangles titres in sera treated with ME. Limits indicate 1 S.E. of the means*

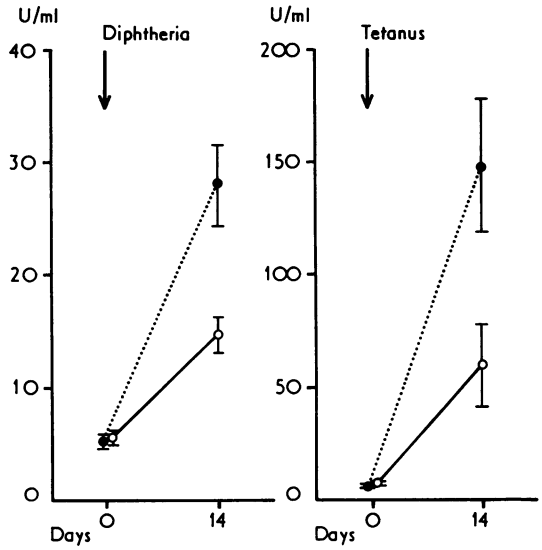

FIG. 3 *Precipitating antiboides to diphtheria and tetanus before and after immunization (↓) in 29 previously vaccinated patients with JRA (closed circles and dotted lines) and their controls (open circles and continuous lines). Limits indicate 1 S.E. of the means. The results are expressed in arbitrary gel-precipitating units (U) per ml.*

having received a second immunization, had more IgG antibodies. The findings in a representative sample of these two types are shown in Fig. 2.

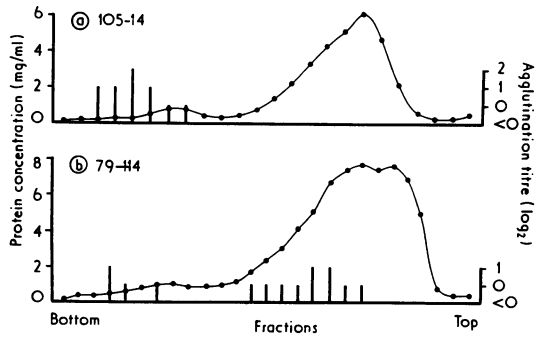

FIG. 2 *Agglutinating activity to* Brucella *(vertical bars) in fractions obtained by density gradient ultracentrifugation of two human sera taken 2 weeks after (a) a single immunization; (b) a second immunization. The amounts applied were 0·1 and 0·2 ml., respectively. Log_2 titres in the untreated whole sera were 11 and 6, and in sera treated with ME 0 and 4, respectively*

SECONDARY IMMUNE RESPONSE

The levels of antibodies to diphtheria and tetanus in the two groups were approximately the same before the present immunization (Fig. 3), although some of the individual values were below the lower limit of detection for the method used. The increase in the amount of antibodies in the 29 pairs of previously vaccinated individuals, was about 2½ times greater in the patient group than in the control group. This was highly significant: $P < 0.01$ (diphtheria) and $P < 0.005$ (tetanus). For these calculations we assumed that values below the limit of detection were the mean of that value and zero. Even assuming that such pre-immunization values in the patient group were at the level of detection and in the control group at zero level, the responses in the patient group were significantly greater than in the control group: $P < 0.02$ (diphtheria) and $P < 0.01$ (tetanus).

IMMUNOGLOBULINS

The patients had significantly higher serum levels of immunoglobulins G, A, and M than the controls, as shown in (Fig. 4, opposite).

The mean IgG ± 2 S.E. was 15·6 ± 2·0 mg./ml. in the patient group and 11·3 ± 1·0 mg./ml. in the control group ($P < 0.001$). The corresponding values for IgA were 1·9 ± 0·4 and 1·3 ± 0·2 mg./ml. ($P < 0.02$) and for IgM 1·3 ± 0·2 and 0·8 ± 0·1 mg./ml. ($P < 0.001$). One patient with a known hypogamma-globulinaemia had very low values of IgG and IgA and a low IgM value.

NATURALLY-OCCURRING ANTIBODIES

No significant difference was found between the levels of anti-A and anti-B haemagglutinins (Table II, opposite).

Neither was there any significant difference in anti-rabbit erythrocyte antibodies between the two groups, the mean log_2 titre ± 2 S.E. being 6·8 ± 0·5 in the patient group and 6·9 ± 0·4 in the control group.

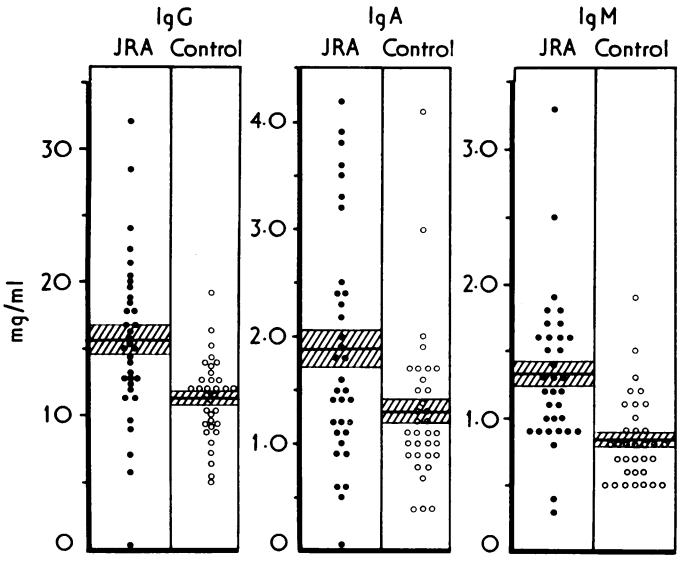

FIG. 4 *Serum immunoglobulins in 34 patients with JRA and their controls. The shaded area indicate 1 S.E. of the means, denoted by solid lines*

Table II *Iso-haemagglutinin titres (log_2) in patients with JRA and in controls*

Blood group/ Agglutinin	*JRA*		*Controls*	
	No.	*Mean ± 2 S.E.*	*No.*	*Mean ± 2 S.E.*
B/anti-A	4	6·3 ± 0·5	2	5·0 ± 2·0
O/anti-A	12	6·4 ± 0·8	8	5·0 ± 0·9
O/anti-B	12	5·6 ± 0·7	8	5·0 ± 1·1
A/anti-B	16	5·2 ± 1·1*	21	5·5 ± 0·7

*=One patient with hypogammaglobulinaemia without anti-B is included.

AUTOANTIBODIES

The mean log_2 titre increased from 2·7 before the immunizations to 3·4 2 weeks after the immunizations, in the patient group, and from 2·5 to 3·0 in the control group. In both groups, sixteen individuals had an increase of the titre, whereas only one had a decrease, giving significance values of $P < 0.001$ and $P < 0.05$, respectively. The number of individuals with a titre above or equal to 1:8 increased by six in the patient group and by one in the control group after the immunization. However, there was no significant difference between the two groups. For rheumatoid factors and immunoconglutinin no such alteration was found.

Discussion

The patient group had a normal primary humoral immune response and a normal level of naturally-occurring antibodies compared with their matched controls. The secondary response and the serum levels of the immunoglobulins IgG, IgA, and IgM, were significantly higher in the patient group than in the control group.

The tests chosen for studying the humoral part of the immunological apparatus in patients with JRA conform with the principles laid out in the WHO report on primary immunodeficiences (Fudenberg and others, 1971). The *Brucella* vaccines used for testing the primary response did not cause side-effects, and had been used in similar studies in patients with RA. Two kinds of *Brucella* vaccines were used due to difficulties with the supply of the Brucellin antigen.

The agglutination method used for titration of *Brucella* antibodies was specific, although *B. melitensis* and *B. suis* cross-reacted with *B. abortus*. Various passive haemagglutination techniques for quantitation of diphtheria and tetanus antibodies failed to give reproducible results in our hands. This difficulty was eliminated by the development of a reversed rocket immunoelectrophoresis technique with a coefficient of variation of 6 to 8 per cent.

It may be argued that, since some of the individuals gave a delayed hypersensitivity response to the *Brucella* antigen (Höyeraal, 1973), they could not have been showing a primary humoral response to the same antigen. Agglutination tests of ME-treated sera and of the fractions obtained by density gradient ultracentrifugation revealed, nevertheless, that the dominating immunoglobulin class was IgM, in conformity with a primary response. This was also shown by Meiselas and others (1961), Waller and others (1966), and Bandilla and others (1970). Further evidence for this was obtained by titrating sera drawn 2 weeks after a second immunization in six individuals. These showed more IgG-specific antibodies.

A possible explanation for the observed discrepancy between cellular and humoral immunity to this antigen is that different antigenic determinants are involved in the two reactions.

The normal primary antibody response to *Brucella* seen in the present study conforms with the observations made in adult patients with RA by Shearn and others (1963) and Waller and others (1966). The high secondary response to diphtheria and tetanus does not conform with any of the reports on similar patients with RA. The observed dissociation between the primary and the secondary antibody response may be similar to the dissociation between the IgM and the IgG response observed in adult patients with RA (Bandilla and others, 1970). This may result from the well-known inhibition of specific IgM antibody production by specific IgG antibody. Another possibility is that primary responses are inhibited more easily than secondary responses by antigen competition between several vaccines given simultaneously, or between vaccines and an unknown antigen. However, in a pilot study, we found no significant difference in the antibody responses whether the two vaccines were given simultaneously or separately.

The immunizations with the killed vaccines gave no serious side-effects and particularly no increased activity of the disease was observed. 2 weeks after immunization no significant increase of rheumatoid factors or immunoconglutinins was found. This conforms with the findings of Shearn and others (1963) and Waller and others (1966), giving only a single dose, whereas Meiselas and others (1961) found a possible increase of rheumatic factors. Bandilla and others (1970) found an apparent correlation between the increase of rheumatoid factor titre and the amount of specific antibody in a group of patients after several immunizations. On the other hand, in the present study, both groups had a significant increase of the titre of pepsin agglutinator. The possible pathogenic significance of a correlation between massive immunization and arthritis is still poorly understood, as reviewed by Aho, Somer, and Salo (1967) and Pettersson (1972).

Possible variations in age and sex should not influence the present results, as responses in matched pairs were compared. No significant correlation was found between the parameters used and the presence of autoantibodies or different forms of treatment. The mean antibody responses, levels of immunoglobulins, naturally-occurring antibodies, and autoantibodies in the ten patients receiving prednisone (average daily dose 7·4 mg.), including two receiving azathioprine (50 mg. daily), did not differ significantly from those of the 24 not receiving such medication. Similar observations were made with regard to the five patients receiving gold therapy, when compared with the 29 not receiving that therapy. This conforms with the observations made by Denman, Denman, Greenwood, Gall, and Heath (1970) in a study mainly of patients with RA, but including some with JRA, although they found a reduction of the titres of rheumatoid factor and levels of immunoglobulins in many patients. Our observations are also consistent with the recent report by Hazleman and Currey (1973) with regard to prednisone and/or azathioprine, but not with regard to gold therapy.

Although the present results suggest no general defect of the humoral part of the immunological apparatus, but rather the contrary, patients with JRA may have hypogammaglobulinaemia, as reported by Good, Rötstein, and Mazzitello (1957). IgA is most often deficient, Cassidy, Burt, Petty, and Sullivan (1969) reporting a frequency of 3·4 per cent. of such patients. Fraser (1969) discussed the association of IgA and autoimmunity. The patient included in the present study with very low serum levels of IgG and IgA conforms with this, although the mean levels of IgA, as well as IgG and IgM, were significantly higher in the patient group than in the control group. Similar elevated serum levels have been reported by Huntley, Thorpe, Lyerly, and Kelsey (1967), Houba and Bardfeld (1969), and Bluestone, Goldberg, Katz, Marchesano, and Calabro (1970), whereas Greenwood (1969) found normal levels in patients with JRA in Nigeria.

Our finding of normal levels of naturally-occurring antibodies does not conform with the lower iso-haemagglutinin titres found in patients with RA (Kornstad, Guldberg, and Kornstad, 1970; Whaley and others, 1971).

It has been suggested (Liew and Parish, 1972) that there is an inverse relationship between the cellular and the humoral parts of the immunological apparatus. Although the delayed hypersensitivity in the present group of patients was found to be impaired (Höyeraal, 1973) and at least some humoral responses were increased, further studies are needed to verify such a possible inverse relationship in patients with JRA.

Summary

Compared with matched healthy individuals, patients with JRA were found to have a normal primary humoral response to *Brucella* antigens and an increased secondary humoral response to immunization of diphtheria and tetanus antigens. Significantly increased serum levels of IgG, IgA, and IgM were found in the patient group, whereas the level of naturally-occurring antibodies (iso-haemagglutinins and antibodies to rabbit erythrocytes) were normal. No adverse clinical effect was observed after the immunizations. Serologically, both groups had a significant increase in the pepsin-agglutinator titre.

Although the results were not consistent, it may thus appear that patients with JRA have a higher activity in the humoral part of the immunological apparatus than controls. Further studies are needed to verify and evaluate the significance of the dissociation of the primary and secondary antibody responses, and the possible inverse correlation between the increased secondary humoral response and the impaired delayed hypersensitivity reactivity observed in these patients.

This work was supported by The Norwegian Research Council for Science and the Humanities and The Norwegian Women's Public Health Organization.

We are indebted to Prof. E. Kåss for his co-operation and to Mrs. M. Hauger for skilled technical assistance. We thank Dr. J. Nagel for the purified diphtheria and tetanus toxoids, and cand. pharm. L. Jelstad, and cand. real. U. Kjennerud for quantitation of diphtheria and tetanus antibodies in representative sera by *in vivo* and other assays *in vitro*.

References

AHO, K., SOMER, T., AND SALO, O. P. (1967) *Proc. Soc. exp. Biol. (N.Y.)*, **124**, 229 (Rheumatoid factor and immuno-conglutinin responses following various vaccinations)

ANSELL, B. M., AND BYWATERS, E. G. L. (1969) 'Juvenile chronic polyarthritis or Still's disease' *in* 'Textbook of the Rheumatic Diseases', ed. W. S. C. Copeman, 4th ed., p. 323. Livingstone, Edinburgh

BANDILLA, K. K., PITTS, N. C., AND MCDUFFIE, F. C. (1970) *Arthr. and Rheum.*, **13**, 214 (Immunoglobulin M deficiency in the immune response of patients with rheumatoid arthritis)

BARR, M., BUCHANAN, W. W., DONIACH, D., AND ROITT, I. M. (1964) *Scot. med. J.*, **9**, 295 (The antibody response to tetanus toxoid in patients with auto-immune thyroiditis)

BLUESTONE, R., GOLDBERG, L. S., KATZ, R. M., MARCHESANO, J. M., AND CALABRO, J. J. (1970) *J. Pediat.*, **77**, 98 (Juvenile rheumatoid arthritis: A serologic survey of 200 consecutive patients)

CASSIDY, J. T., BURT, A., PETTY, R., AND SULLIVAN, D. (1969) *New Engl. J. Med.*, **280**, 275 (Selective IgA deficiency in connective tissue diseases)

COOMBS, R. R. A., COOMBS, A. M., AND INGRAM, D. G. (1961) 'The Serology of Conglutination and its Relation to Disease'. Blackwell Scientific Publications, Oxford

CREGER, W. P., CHOY, S. H., AND RANTZ, L. A. (1951) *J. Immunol.*, **66**, 445 (Experimental determination of the hypersensitive diathesis in man)

DENMAN, E. J., DENMAN, A. M., GREENWOOD, B. M., GALL, D., AND HEATH, R. B. (1970) *Ann. rheum. Dis.*, **29**, 220 (Failure of cytotoxic drugs to suppress immune responses of patients with rheumatoid arthritis)

DIEM, K. (1962) 'Documenta Geigy. Scientific Tables', 6th ed. Geigy, Basle

FRASER, K. J. (1969) *Lancet*, **2**, 804 (IgA immunoglobulins and autoimmunity)

FUDENBERG, H. H., AND KUNKEL, H. G. (1957) *J. exp. Med.*, **106**, 689 (Physical properties of the red cell agglutinins in acquired hemolytic anemia)

——, GOOD, R. A., GOODMAN, H. C., HITZIG, W., KUNKEL, H. G. (Chairman), ROITT, I. M., ROSEN, F. S., ROWE, D. S., SELIGMANN, M., AND SOOTHILL, J. R. (1971) *Pediatrics*, **47**, 927 (Primary immunodeficiencies. Report of a World Health Organization Committee)

GOOD, R. A., RÖTSTEIN, J., AND MAZZITELLO, W. F. (1957) *J. Lab. clin. Med.*, **49**, 343 (The simultaneous occurrence of rheumatoid arthritis and agammaglobulinemia)

GREENWOOD, B. M. (1969) *Ann. rheum. Dis.*, **28**, 617 (Polyarthritis in Western Nigeria. II. Still's disease)

GREENWOOD, R., AND BARR, M. (1960) *Ann. phys. Med.*, **5**, 258 (Circulating antibody production in rheumatoid arthritis)

HALL, W. H., AND MANION, R. E. (1953) *J. clin. Invest.*, **32**, 96 (Comparison of the Coombs test with other methods for brucella agglutinins in human serum)

HAZLEMAN, B. L., AND CURREY, H. L. F. (1973) *Ann. rheum. Dis.*, **32**, 276 (Antibody-producing capacity in rheumatoid arthritis)

HOUBA, V., AND BARDFELD, R. (1969) *Ibid.*, **28**, 55 (Serum immunoglobulins in juvenile rheumatoid arthritis)

HUNTLEY, C. C., THORPE, D. P., LYERLY, A. D., AND KELSEY, W. M. (1967) *Amer. J. Dis. Child.*, **113**, 411 (Rheumatoid arthritis with IgA deficiency)

HÖYERAAL, H. M. (1973) *Ann. rheum. Dis.*, **32**, 331 (Impaired delayed hypersensitivity in juvenile rheumatoid arthritis)

——, VANDVIK, B., AND MELLBYE, O. J., to be published (Immunochemical quantitation of diphtheria and tetanus antibodies by reversed rocket immunoelectrophoresis)

KORNSTAD, L., GULDBERG, D., AND KORNSTAD, A. M. G. (1970) *Ann. rheum. Dis.*, **29**, 421 (Isohaemagglutinins anti-A and anti-B in rheumatoid arthritis and ankyloisng spondylitis)

LARSEN, R. A., AND BØYUM, A. (1972) *Acta med. scand.*, **543**, Suppl. 21 (Family studies in systemic lupus erythematosus (SLE). II. Development of an indirect LE factor test)

LIEW, F. Y., AND PARISH, C. R. (1972) *Cell. Immunol.*, **4**, 66 (Regulation of the immune response by antibody I. Suppression of antibody formation and concomitant enhancement of cell-mediated immunity by passive antibody)

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., AND RANDALL, R. J. (1951) *J. biol. Chem.*, **193**, 265 (Protein measurement with the Folin phenol reagent)

MANCINI, G., CARBONARA, A. O., AND HEREMANS, J. F. (1965) *Immunochemistry*, **2**, 235 (Immunochemical quantitation of antigens by single radial immunodiffusion)
MEISELAS, L. E., ZINGALE, S. B., LEE, S. L., RICHMAN, S., AND SIEGEL, M. (1961) *J. clin. Invest.*, **40**, 1872 (Antibody production in rheumatic diseases. The effect of brucella antigen)
MELLBYE, O. J., AND NATVIG, J. B. (1970) *Clin. exp. Immunol.*, **7**, 257 (Experimentally produced antibodies to the pepsin site of IgG due to untreated autologous IgG in immune complexes)
MUNTHE, E. (1970) *Acta rheum. scand.*, **16**, 240 (Relationship between IgG complexes and anti-IgG antibodies in rheumatoid arthritis)
PETTERSSON, T. (1972) *Scand. J. Rheum.*, **1**, 61 (Immunization and arthritis)
RAWSON, A. J., ABELSON, N. M., AND MCCARTY, D. J. (1961) *Arthr. and Rheum.*, **4**, 463 (The isohemagglutinin partition in rheumatoid arthritis)
SHEARN, M. A., EPSTEIN, W. V., AND ENGLEMAN, E. P. (1963) *Proc. Soc. exp. Biol. (N.Y.)*, **113**, 1001 (Antibody response to brucella antigen in patients with rheumatoid arthritis)
VAUGHAN, J. H., AND BUTLER, V. P. (1962) *Ann. intern. Med.*, **56**, 1 (Current status of the rheumatoid factor)
WALLER, M., ELLMAN, H. M., AND TOONE, E. C. (1966) *Acta rheum. scand.*, **12**, 250 (Brucella immunization in patients with seropositive and seronegative rheumatoid arthritis)
WHALEY, K., GLEN, A. C. A., MACSWEEN, R. N. M., DEODHAR, S., DICK, W. C., NUKI, G., WILLIAMSON, J., AND BUCHANAN, W. W. (1971) *Clin. exp. Immunol.*, **9**, 721 (Immunological responses in Sjögren's syndrome and rheumatoid arthritis)