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JUVENILE RHEUMATOID ARTHRITIS

CELLULAR HYPERSENSITIVITY AND SELECTIVE IgA DEFICIENCY

R. S. PANUSH, N. E. BIANCO, P. H. SCHUR, R. E. ROCKLIN, J. R. DAVID AND J. S. STILLMAN

Department of Medicine, Harvard Medical School, at the Robert Breck Brigham Hospital, Boston, Massachusetts

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SUMMARY

Although humoral immune mechanisms are currently thought to be of pathogenetic significance in juvenile rheumatoid arthritis (JRA), little is known about the role of cellular hypersensitivity in this disease. A possible association between abnormalities of humoral and cellular immunity exists in patients with ataxia-telangiectasia, who may have absent IgA, abnormal delayed hypersensitivity, or both. As IgA deficiency has been noted in 2–3% of patients with JRA, we have studied selected aspects of humoral and cellular hypersensitivity in patients with JRA and IgA deficiency and in patients with JRA and normal IgA levels. All patients had normal serum levels of complement, IgG, IgM, and IgD.

Cellular hypersensitivity was evaluated by cutaneous delayed-type hypersensitivity, *in vitro* migration inhibitory factor production, and antigen induced 3H-thymidine incorporation by lymphocytes using Candida and Streptokinase–Streptodornase antigens. Two of four IgA deficient patients had positive *in vitro* but negative *in vivo* responses to antigens. Seven of fourteen JRA patients with normal immunoglobulin levels exhibited a similar dissociation of *in vivo* and *in vitro* manifestations of delayed hypersensitivity. This pattern of cellular immune response was associated with activity and chronicity of disease; it was independent of IgA deficiency.

INTRODUCTION

Recent studies suggest that humoral immune responses are responsible, in part, for the inflammatory synovitis observed in patients with juvenile rheumatoid arthritis (JRA). Rheumatoid factors may interact with γ-globulin or immune complexes to activate the complement system, which can then initiate many of the resulting inflammatory changes. Scant information, however, is available regarding the possible role of cellular immune responses in this disease.

Abnormalities of both cellular and humoral immunity have been noted in patients with

Correspondence: Dr P. H. Schur, Department of Medicine, Robert Brigham Hospital, Boston, Massachusetts 02120, U.S.A.

ataxia-telangiectasia. In these patients both selective IgA deficiency and impaired delayed hypersensitivity may co-exist (Peterson & Good, 1968). Recently, selective IgA deficiency has been described in patients with rheumatic diseases, particularly JRA (Claman, Hartley & Merrill, 1966; Huntley *et al.*, 1967; Cassidy & Burt, 1968; Heremans & Crabbe, 1968; Tomasi, 1968). Aside from a brief report of impaired lymphocyte transformation to phytohaemagglutinin in IgA deficient patients with 'connective tissue disease' (Cassidy *et al.*, 1969a) and reports of normal delayed hypersensitivity in IgA deficient patients (Ammann & Hong, 1970; Ammann & Hong, 1971), little else is known about cellular immune function in patients with either JRA or IgA deficiency.

This study reports observations of certain aspects of cellular immune function in patients with JRA and IgA deficiency and compares them to findings in patients with JRA and normal IgA levels. A pattern of cellular immune response characterized by depressed skin reactivity to one or more antigens but evidence of *in vitro* cellular hypersensitivity to the same antigens was frequently found in those patients with chronic disease. This particular pattern of cellular immune response was only rarely seen in patients who were in remission and was not related to IgA deficiency.

MATERIALS AND METHODS

Patient Selection

When immunoglobulin levels were determined on a large group of patients, three of 176 patients with JRA and one of 161 patients with RA were found to have selective IgA deficiency. The diagnostic criteria of Brewer (1970) and Ropes *et al.* (1959) were used to confirm the diagnoses of JRA and RA respectively. These four patients with IgA deficiency along with fourteen consecutive patients admitted to the hospital with JRA were studied. These fourteen patients all had normal IgA levels.

Clinical evaluation

Clinical evaluation was performed as previously described (Panush, Bianco & Schur, 1971). Severity of articular involvement was estimated as follows: A normal joint examination was scored as 0; involvement of one or two joints was scored as +, three to five joints as ++, and six or more joints as +++. Radiologic findings of erosions, joint narrowing, degenerative changes, ankylosis, dislocation, subluxation, or osteoporosis were recorded and similarly graded as 0 to +++. Use of salicylates, corticosteroids, antimalarial drugs, gold salts, or indomethacin was tabulated. Patients were evaluated for functional capacity according to the criteria of Lansbury (1967). Disease course was characterized as remitting or chronic as described by Sharp *et al.* (1964). Routine laboratory studies on all patients included complete blood count, differential count, blood sedimentation index (Rourke & Ernstene, 1930), serum protein electrophoresis, LE cell preparation, antinuclear antibody test (Gonzalez & Rothfield, 1966), and latex fixation test (Hall, Mednis & Bayles, 1958).

Immunoglobulin and complement studies

Serum levels of total IgG, IgG1, IgG2, IgG3, IgG4, IgA, IgM, and IgD were measured by radial immunodiffusion (Mancini, Carbonara & Heremans, 1965; Schur *et al.*, 1970).

Serum IgE levels were kindly determined by Dr Edward Spitz by radio-immunodiffusion (Ishizaka *et al.*, 1970).

Total serum haemolytic complement (CH50) levels were assayed by the method of Kent & Fife (1963). Normal levels for immunoglobulin and complement determinations are listed in Table 1.

TABLE 1. Delayed hypersensitivity responses, complement and immunoglobulin levels

Patient	Delayed hypersensitivity testing* SKSD	Candida	CH50 u/ml	IgG mg/ml	IgA mg/ml	IgM mg/ml	IgD mg/ml	IgE ng/ml
ML†	III	Ib	273	13·0	<0·005†	1·70	0·027	<25
JM†	II	II	173	10·5	<0·005†	1·08	0·072	<25
MCh	Ia	II	211	8·0	1·14	0·90	0·033	<25
RS	III	Ib	124	8·0	1·66	1·00	0·100	230
ET	II	Ib	235	14·0	1·40	1·90	0·076	80
BR	II	Ib	317	6·0	2·80	1·00	0·035	<25
JJ	III	III	127	40·0	2·20	3·20	0·031	85
DH	III	Ib	200	8·0	0·84	1·50	0·035	60
CH	Ib	II	188	10·0	1·26	1·20	0·036	65
MM†	Ia	Ib	173	9·1	<0·005†	0·58	0·025	25
CC†	Ia	Ib	185	11·2	<0·005†	1·86	0·025	70
BD	Ib	Ib	254	14·2	1·26	2·60	0·176	60
JS	Ia	Ia	167	12·0	3·60	1·00	0·110	780
CP	Ia	Ia	148	8·4	1·50	0·80	0·045	25
SC	Ia	Ia	228	24·0	3·20	1·50	0·035	150
SP	Ia	Ia	256	9·0	3·20	1·55	0·032	65
LK	Ia	Ia	259	12·0	1·50	1·65	0·100	70
MC‡	Ia		238	18·0	2·40	1·45	0·028	45
Normal§	Ia or Ib	Ia or Ib	200 ±50	16·2 ±7·7	2·80 ±2·00	1·50 ±0·90	0·065 ±0·100	167 ±270

* Patterns of delayed hypersensitivity testing are defined in Table 2.
† Denotes the four patients with IgA deficiency.
‡ MC did not undergo delayed hypersensitivity testing to Candida antigen.
§ Normal mean values ± 2 standard deviations for this laboratory.

In addition, the four sera from patients deficient in IgA were examined for the presence of antibodies to IgA by double diffusion in agar (Ouchterlony, 1962) in a system sensitive to 0·2 mg/ml antibody.

Delayed hypersensitivity testing

Cellular immune function was evaluated by testing for cutaneous delayed-type hypersensitivity, *in vitro* migration inhibitory factor (MIF) production by autologous lymphocytes, and 3H-thymidine incorporation by lymphocytes in response to antigens. *Candida albicans* antigen* and Streptokinase-Streptodornase† were used for both *in vivo* and *in vitro*

* Dermatophyton 'O', Hollister-Stier, Yeadon, Pennsylvania.
† Varidase, Lederle Laboratories, Pearl River, New York.

testing as outlined (Rocklin, Meyers & David, 1970). The method for the production of MIF by human lymphocytes has been previously described (Rocklin, Meyers & David, 1970). Briefly, heparinized blood from patients studied was sedimented. The leucocyte-rich plasma was collected and the lymphocytes were separated from polymorphonuclear leucocytes and monocytes by passage through cotton columns. After several washes, the cell concentration was adjusted to 3×10^6 cells/ml. The lymphocytes were cultured with or without antigen in serum free medium TC 199 (Microbiological Associates, Bethesda, Maryland). Cell free supernatants were harvested daily for 3 days, pooled, concentrated, and assayed for MIF activity on guinea-pig macrophages in capillary tubes (David *et al.*, 1964). After determining the area of migration, the % migration was calculated from the following formula:

$$\% \text{ migration} = X/Y \times 100$$

$$X = \frac{\text{Area of migration in supernatant with antigen}}{\text{Area of migration in media with antigen}}$$

$$Y = \frac{\text{Area of migration in supernatant without antigen}}{\text{Area of migration in media without antigen}}$$

In previous studies a migration of less than 80% was significant and indicated the production of MIF (Rocklin, Meyers & David, 1970). This criterion was employed in the present study.

Antigen-induced 3H-thymidine incorporation was measured by culturing $1{\cdot}5 \times 10^6$ lymphocytes in 1·5 ml of TC 199 medium containing 15% autologous plasma with or without antigen for 6 days (Rocklin *et al.*, 1970c). Three hours prior to terminating the cultures, 0·5 μCi of 3H-thymidine (specific activity 5·0 mCi/mmole, Amersham-Searle, Des Plaines, Illinois) were added to each tube. The deoxyribonucleic acid labelled material was obtained by trichloroacetic acid precipitation, washed with methanol, and solubilized with sodium hydroxide. The counts per minute (cpm) of duplicate cultures were determined by scintillation counting. When the incorporation of 3H-thymidine in antigen stimulated cultures was three or more times that of unstimulated cultures, the test was considered positive.

The results of these three assays—skin tests, MIF production, and incorporation of 3H-thymidine in response to the two antigens—divided the patients into three groups (Table 2). In group I, all three parameters of cellular reactivity were either positive (Ia) or negative (Ib). In group II, dermal hypersensitivity and MIF production were negative, but the lymphocytes from these patients showed increased incorporation of 3H-thymidine in response to antigenic stimulation. In group III, the skin test results were negative, but lymphocytes from these patients incorporated 3H-thymidine and made MIF in response to antigens. Normal individuals previously studied all fell into groups Ia or Ib, i.e. their *in vivo* responses to antigens paralleled their *in vitro* responses (Rocklin *et al.*, 1970c).

RESULTS

Immunoglobulin levels

Immunoglobulin levels for the patients studied are summarized in Table 1. Four patients (ML, JM, MM, CC) had serum IgA levels less than 0·005 mg/ml, the lower limit of sensitivity

TABLE 2. Summary of *in vivo* and *in vitro* cellular immune responses

SKSD					Candida				
Group	No. patients	Skin test	MIF	3H-thymidine	Group	No. patients*	Skin test	MIF	3H-thymidine
Ia	10	+	+	+	Ia	5	+	+	+
Ib	2	–	–	–	Ib	7	–	–	–
II	2	–	–	+	II	4	–	–	+
III	4	–	+	+	III	1	–	+	+

* One patient (MC) was not tested to Candida antigen.

of the antiserum. Further, precipitating antibodies to IgA were not detected in sera from those four patients. Levels of IgG, IgG1, IgG2, IgG3, IgG4, IgM and IgD were normal or, in some instances, slightly elevated in all patients. IgE levels were variable among all patients studied but were generally similar to levels measured in normal individuals. Aside from IgA, levels of immunoglobulins were comparable in all patients.

Complement levels

Serum CH50 activities for the eighteen patients studied are listed in Table 1. Complement levels were similar in the groups of four IgA deficient patients and fourteen patients with normal immunoglobulin levels. Further, complement levels for all these patients were comparable to levels described in a series of 147 patients with JRA (Bianco *et al.*, 1971).

Cellular hypersensitivity

Table 2 summarizes the three tests of cellular reactivity examined: the skin tests, MIF production, and incorporation of 3H-thymidine in response to the two antigens. The results of these assays divide the patients into three groups, as explained, the majority of patients being in groups I and II.

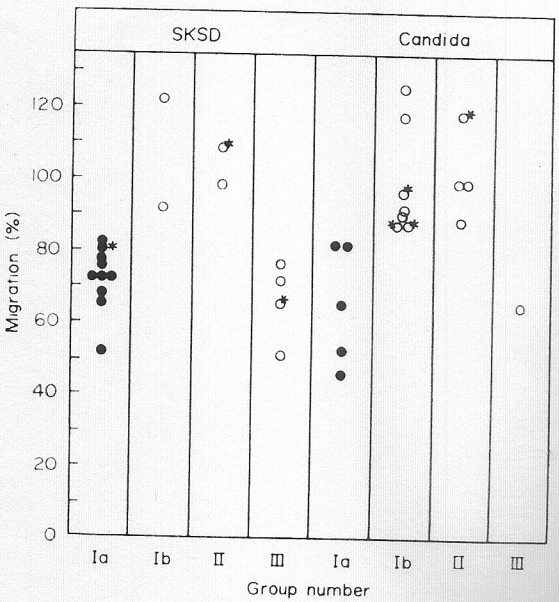

FIG. 1. Results of MIF assays. Open circles represent patients with negative skin tests while closed circles indicate patients with positive skin tests. Asterisks denote patients with IgA deficiency. Groups Ia, Ib, II, and III are defined in Table 2. One patient (MC) was not tested to Candida antigen.

The individual results of the MIF assay are shown in Fig. 1. Lymphocytes from patients in group I with positive skin tests (Ia) usually made MIF in response to antigens. Lymphocytes from three of these patients (JS, LK, and MCh) gave borderline negative MIF results (82%, 83%, and 83% respectively). Occasionally cells from skin test negative individuals do

not make detectable MIF on a single determination (Rocklin, Meyers & David, 1970b). However, because these three patients had positive skin tests and antigen induced 3H-thymidine incorporation, they were grouped Ia. Lymphocytes from patients in group I with negative skin tests (Ib) failed to make MIF. Lymphocytes from patients in group II, with negative skin tests, did not produce MIF but responded to antigen induced 3H-thymidine incorporation. However, lymphocytes from patients in group III, with negative skin tests, did produce MIF in response to antigenic stimulation.

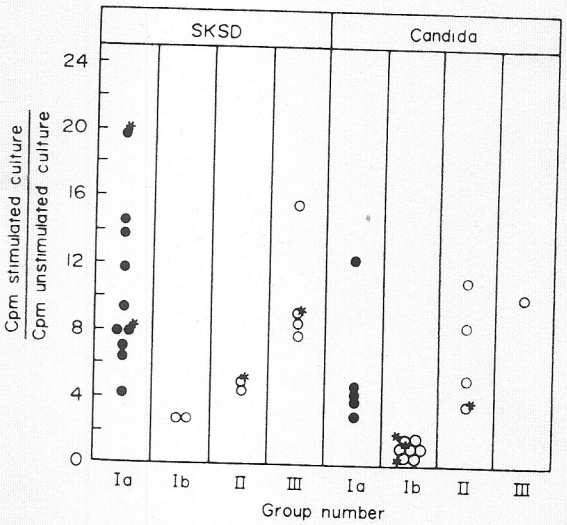

FIG. 2. Results of 3H-thymidine incorporation. Open circles represent patients with negative skin tests while closed circles indicate patients with positive skin tests. Asterisks denote patients with IgA deficiency. Groups Ia, Ib, II and III are defined in Table 2. One patient (MC) was not tested to Candida antigen.

The individual results of 3H-thymidine incorporation by lymphocytes are seen in Fig. 2. Lymphocytes from patients in group I with positive skin tests (Ia) incorporated 3H-thymidine normally; those in group I with negative skin tests (Ib) did not. In contrast, lymphocytes from patients in groups II and III, with negative skin tests, had increased 3H-thymidine incorporation in response to antigens. The amount of 3H-thymidine incorporated by lymphocytes from these patients was similar to that observed in normal individuals.

Clinical correlations

In order to learn if delayed hypersensitivity responses were impaired in patients with IgA deficiency and rheumatoid arthritis, four patients with absent serum IgA were studied (Tables 1 and 3). Two patients (MM and CC) had normal *in vitro* and *in vivo* responses and were in remission from disease. Two patients (ML and JM) had a dissociation of *in vitro* and *in vivo* cellular immune responses and had chronic active disease. ML had consistently negative skin tests to Candida and SKSD but evidence of sensitized cells *in vitro*, by MIF and 3H-thymidine incorporation, in response to SKSD. Similarly, JM had persistently negative skin tests and negative MIF to both antigens but her lymphocytes incorporated

TABLE 3. Delayed hypersensitivity responses and clinical studies

Patient	Age and sex	Delayed hypersensitivity testing*		Disease duration (years)	American rheumatism association functional class	Course	Joint involvement		Drugs‡
		SKSD	Candida				Clinical	Radiological	
ML†§	58 F	III	Ib	8	III	Chronic	+++	++	A, AM
JM†	17 F	II	II	9	II	Chronic	++	++	A, AM
MCh	16 M	Ia	II	5	III	Chronic	++	+	A, S
RS	37 M	III	Ib	23	II	Remit	±	+	A
ET	23 M	II	Ib	13	II–III	Chronic	++	++	I
BR	19 F	II	Ib	5	II–III	Chronic	++	+	A, G
JJ	20 F	III	III	12	III	Chronic	+++	++	A, AM
DH	21 F	III	Ib	7	II	Chronic	++	+	A
CH	29 F	Ib	II	20	III	Chronic	+++	++	A
MM†	17 F	Ia	Ib	7	I	Remit	±	+	A
CC†	17 F	Ia	Ib	6	I	Remit	0	0	None
BD	5 M	Ib	Ib	1/2	I	Remit	0	0	None
JS	16 F	Ia	Ia	2	II	Remit	+	+	A, AM
CP	21 F	Ia	Ia	10	I	Remit	0	0	None
SC	26 F	Ia	Ia	19	II	Chronic	++	+	A
SP	17 F	Ia	Ia	11	I	Remit	0	0	A
LK	20 F	Ia	Ia	8	I	Remit	0	0	A
MC¶	17 F	Ia		2	II	Chronic	++	±	A, AM
Normal		Ia or Ib	Ia or Ib						

* Patterns of delayed hypersensitivity testing are defined in Table 2.
† Denotes the four patients with IgA deficiency
‡ A = acetylsalicylic acid; AM = antimalarials, G = gold salts; S = steroids; I = indomethacin
§ ML was the only patient studied who had adult onset rheumatoid arthritis.
¶ MC did not undergo delayed hypersensitivity testing to Candida antigen.

thymidine normally *in vitro* in response to these antigens. The negative skin tests in these two patients could not be attributed to decreased numbers of circulating lymphocytes, for the patients had 1740 and 2190 lymphocytes per mm^3 respectively. Thus no constant association was observed between IgA deficiency and delayed hypersensitivity responses. The four patients with IgA deficiency did not have recurrent infections.

It was of interest that seven of fourteen patients with normal IgA levels also had dissociation of *in vivo* and *in vitro* cellular immune responses (type II or III, Table 3), while seven other patients exhibited no dissociation (type Ia or Ib). It is of note that those patients with a dissociation of cellular immune responses (type II or III) had a longer disease duration, greater functional impairment, chronic disease course, and more evidence of clinical and radiological articular involvement.

The patients with dissociated *in vitro* and *in vivo* delayed hypersensitivity responses and chronic disease, as expected, were taking more medication than patients with normal delayed hypersensitivity testing and remission from disease (Table 1). Of the nine patients with type II or III cellular immune responses, eight were taking acetylsalicylic acid, three were using antimalarial drugs, one was receiving gold salt injections, one was taking steroids, and one patient was on indomethacin. Of the nine patients with type I responses, six were taking acetylsalicylic acid, two were using antimalarial drugs, and three were off all medications.

The possibility that low peripheral lymphocyte counts might have been responsible for the negative skin tests in patients with type II or III responses was considered. Only one patient (DH), however, with negative skin tests and positive *in vitro* assays had a lymphocyte count of less than 1400 cells per mm^3 (756 cells per mm^3). In addition, patients MC (861 cells per mm^3), JS (1235 cells per mm^3), and LK (1300 cells per mm^3) had reduced numbers of circulating lymphocytes but positive *in vivo* and *in vitro* testing (type Ia responses).

DISCUSSION

This study has shown that a dissociation of *in vivo* and *in vitro* cellular immune responses, characterized by negative skin test but positive migration inhibitory factor or 3H-thymidine incorporation assays to common antigens, occurred in patients with JRA. This pattern of response was strongly associated with chronicity of illness irrespective of IgA deficiency.

Selective IgA deficiency is probably the most common selective immunoglobulin deficiency and has received considerable attention. It has been found in less than one of 500 apparently normal individuals (Cassidy & Burt, 1968; Johansson, Hogman & Killander, 1968; Bachmann, 1965; Hanson, 1968). An increased incidence of this immunodeficiency in patients with a variety of illnesses has been reported and reviewed (Heremans & Crabbe, 1968; Tomasi, 1968; Ammann & Hong, 1970; Ammann & Hong, 1971). Of particular interest has been the finding of IgA deficiency among patients with rheumatoid arthritis (Cassidy & Burt, 1967; Cassidy & Burt, 1968; Claman, Hartley & Merrill, 1966; Huntley *et al.*, 1967). The incidence of IgA deficiency in patients with JRA has been 2–3% (Cassidy & Burt, 1968; Bluestone *et al.*, 1970). Antibodies directed against IgA have been described in many individuals with IgA deficiency (Cassidy *et al.*, 1969b; Vyas *et al.*, 1969; Ammann & Hong, 1970). Concommitant IgA and IgE deficiency has been described in patients with with recurrent sinopulmonary infections and ataxia-telangiectasia (Ammann *et al.*, 1969).

In the present study four patients with rheumatoid arthritis and IgA deficiency were

identified. Three of these patients had juvenile onset and one had adult onset RA. Our incidence of IgA deficiency in JRA, nearly 2%, is in accord with other studies. Our four patients did not have demonstrable precipitating antibodies to IgA. Haemagglutinating antibodies to IgA (Vyas *et al.*, 1969) may not have been recognized in our test system. Of interest is the observation that two patients with IgA deficiency did not have detectable levels of serum IgE. These two patients had chronic arthritis and dissociation of *in vivo* and *in vitro* responses to antigens. The other two patients with IgA deficiency had detectable serum IgE, parallel *in vivo* and *in vitro* responses to antigens, and remission from disease. However, since normal individuals and other patients with JRA occasionally do not have detectable serum IgE, the significance of this observation is unknown.

Cellular immunity has been carefully investigated in patients with IgA deficiency and ataxia-telangiectasia. These patients frequently have negative skin tests to common antigens, cannot be sensitized to dinitrochlorobenzene, and do not reject homografts normally (Peterson & Good, 1968). There are only sporadic reports of the function of the cellular immune system in other patients with selective IgA deficiency. Goldberg, Barnett & Fudenberg (1968) described an adult with IgA deficiency who had positive intracutaneous tests to Candida, mumps, purified protein derivative, and a normal lymphocyte response to phytohaemagglutinin (PHA). Cassidy & Burt (1968) have reported normal skin test results in patients with JRA and IgA deficiency, but have subsequently indicated that IgA deficient patients with 'connective tissue disease,' have impaired lymphocyte transformation to PHA (Cassidy *et al.*, 1969a). Ammann & Hong (1971), in thirty patients with selective IgA deficiency, found normal cutaneous delayed hypersensitivity, lymphocyte responses to PHA, and small lymphocyte counts.

Studies of cellular immunity in rheumatoid arthritis have produced inconsistent results. Investigations of patients with RA have demonstrated diminished susceptibility to contact sensitization (Epstein & Jessary, 1959; Whaley *et al.*, 1970), reduced incidence of positive skin tests to common antigens (Whaley *et al.*, 1970; Hayes, Ward & Jennings, 1970), diminished lymphocyte reactivity in one-way mixed lymphocyte cultures (Astorga & Williams, 1969), impaired *in vivo* and *in vitro* responses to PHA and streptolysin O (Leventhal Waldorf & Talal, 1967), both increased and decreased globulin synthesis by peripheral lymphocytes (Forbes & Henderson, 1966), and increased lymphocyte transformation to PHA (La Polla & Thurmon 1966). Anti-γ-globulins of the 19S type were found capable of inducing transformation of human lymphocytes (Messner *et al.*, 1969) but IgG failed to cause transformation of rheumatoid lymphocytes (Kacaki, Bullock & Vaughan, 1969). Depressed lymphocyte responses to PHA were described in patients with ankylosing spondylitis, particularly among patients with symptomatic disease (Escanilla, Alepa & Reefe, 1970). Impaired lymphocyte transformation in some patients with 'connective tissue disease' has also been noted (Cassidy *et al.*, 1969a).

In a preliminary report, Lockshin & Rounsaville (1969) found that skin tests and MIF production paralleled each other in normal individuals and patients with rheumatic diseases. It would have been of interest to know which of their patients gave the occasional 'false positive' results since these patients would have been similar to our group III.

Sukernick, Hanin & Mosolov (1968) have shown that peripheral lymphocytes from patients with RA caused severe damage to monolayers of human fibroblasts. Loewi (1969) has shown that 'a reaction of delayed hypersensitivity could produce inflammation in the synovium' of experimental animals.

The recent study demonstrates a pattern of dissociation of *in vivo* and *in vitro* cellular immune responses in half of the patients studied, irrespective of IgA deficiency. There was a strong association between this particular pattern of cellular immune response and chronicity of disease. Similar dissociations of cellular immune responses in certain patients with chronic mucocutaneous candidiasis and certain other chronic diseases have been noted previously (Chilgren *et al.*, 1969; Rocklin *et al.*, 1970a; Rocklin *et al.*, 1970c). Although impaired cutaneous delayed type hypersensitivity has been recognized in patients with RA (Whaley *et al.*, 1970; Hayes, Ward & Jennings, 1970), it has not been appreciated that these patients may have *in vitro* evidence of sensitized lymphocytes. The possible mechanism producing these dissociated responses has been discussed in detail elsewhere (Rocklin *et al.*, 1970c). It was possible to exclude low peripheral lymphocyte counts as responsible for the instances of cutaneous anergy in patients with *in vitro* evidence of sensitized cells. The possibility, however, that the slightly increased amounts of drugs used by patients with chronic disease may have influenced the manifestations of delayed hypersensitivity cannot be totally excluded. Nonetheless it is clear that a group of patients with persistent disease and changes in the pattern of cellular immune responses exists. Whether such changes predispose these children to chronic arthritis or merely result from indolent disease is not known.

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REFERENCES

AMMANN, A.J., CAIN, W.A., ISHIZAKA, K., HONG, R. & GOOD, R.A. (1969) Immunoglobulin E deficiency in atazia-telangiectasia. *New Engl. J. Med.* **281,** 469.

AMMANN, A.J. & HONG, R. (1970) Selective IgA deficiency and autoimmunity. *Clin. exp. Immunol.* **7,** 833.

AMMANN, A.J. & HONG, R. (1971) Selective IgA deficiency: presentation of 30 cases and a review of the literature. *Medicine (Baltimore),* **50,** 223.

ASTORGA, G.P. & WILLIAMS, R.C. (1969) Altered reactivity in mixed lymphocyte culture of lymphocytes from patients with rheumatoid arthritis. *Arthr. and Rheum.* **12,** 547.

BACHMANN, R. (1965) Studies on the serum γ1A-globulin level III. The frequency of a-γ1A-globulinemia. *Scand. J. Clin. Lab. Invest.* **17,** 316.

BIANCO, N.E., PANUSH, R.S., STILLMAN, J.S. & SCHUR, P.H. (1971) Immunologic studies of juvenile rheumatoid arthritis. *Arth. and Rheum.* (In press).

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

BREWER, E.J. (1970) Differential diagnosis. *Juvenile rheumatoid arthritis* (Ed. by E. J. Brewer), p. 80. W. B. Saunders Co., Philadelphia.

CASSIDY, J.T. & BURT, A. (1967) Isolated IgA deficiency in juvenile rheumatoid arthritis. *Arthr. and Rheum.* **10,** 272.

CASSIDY, J.T. & BURT, A. (1968) Selective hypogammaglobulinemia in rheumatoid arthritis. *Univ. Mich. Med. Center J.* **34,** 241.

CASSIDY, J.T., FRIEDLANDER, R., SCHMICKEL, R. PETTY, R. & SULLIVAN, D. (1969a) Impaired lymphocyte transformation in patients with connective tissue disease (CTD) and selective immunoglobulin A (IgA) deficiency. *Arthr. and Rheum.* **12,** 659.

CASSIDY, J.T., PETTY, R., BURT, A. & SULLIVAN, D. (1969b) Anti-IgA antibodies in patients with selective IgA deficiency and connective tissue disease. *Clin. Res.* **27,** 351.

CHILGREN, R.A., MEUWISSEN, H.J., QUIE, P.G., GOOD, R.A. & HONG, R. (1969) The cellular immune defect in chronic mucocutaneous candidiasis. *Lancet,* **i,** 1286.

CLAMAN, H.N., HARTLEY, T.F. & MERRILL, D. (1966) Hypogammaglobulinemia, primary and secondary: immunoglobulin levels (γG, γA, γM) in one hundred and twenty-five patients. *J. Allergy,* **38,** 215.

DAVID, J.R., AL-ASKARI, S., LAWRENCE, H.S. & THOMAS, L. (1964) Delayed hypersensitivity *in vitro*. I. The specificity of inhibition of cell migration by antigens. *J. Immunol.* **93,** 264.

EPSTEIN, W.L. & JESSAR, R.A. (1959) Contact-type delayed hypersensitivity in patients with rheumatoid arthritis. *Arthr. and Rheum.* **2,** 178.

ESCANILLA, F., ALEPA, F.P. & REEFE, W. (1970) Impaired lymphocyte responses in ankylosing spondylitis. *Arthr. and Rheum.* **13,** 314.

FORBES, I.J. & HENDERSON, D.W. (1966) Globulin synthesis by human peripheral lymphocytes. *In vitro* measurements using lymphocytes from normals and patients with disease. *Ann. intern. Med.* **65,** 69.

GOLDBERG, L.S., BARNETT, H.V. & FUNDENBERG, H.H. (1968) Selective absence of IgA: a family study. *J. Lab. clin. Med.* **72,** 204.

GONZALEZ, E.N. & ROTHFIELD, N.E. (1966) Immunoglobulin class and pattern of nuclear fluorescence in systemic lupus erythematosus. *New Engl. J. Med.* **274,** 1333.

HALL, A.P., MEDNIS, A.D. & BAYLES, T.B. (1958) The latex agglutination and inhibition reactions. Clinical experience in the diagnosis of rheumatoid arthritis. *New Engl. J. Med.* **258,** 731.

HANSON, L.A. (1968) Aspects of the absence of the IgA system. *Immunologic deficiency diseases in man* (Ed. by D. Bergsma), p. 298. National Foundation March of Dimes, New York.

HAYES, J.R., WARD, D.J. & JENNINGS, J.F. (1970) Studies on cell-mediated hypersensitivity responses in rheumatoid arthritis. *Proceedings of the third symposium on impaired cell-mediated hypersensitivity in man* (Ed. by J.F. Jennings and D.J. Ward), p. 37, The Robert Jones and Agnes Hunt Orthopaedic Hospital Management Committee, Oswestry, England.

HEREMANS, J.F. & GRABBE, P.A. (1968) IgA deficiency: general considerations and relation to human disease. *Immunologic deficiency diseases in man* (Ed. by D. Bergsma), p. 298. National Foundation March of Dimes, New York.

HUNTLEY, G.C., THORPE, D.P., LYERLY, A.D. & KELSEY, W.M. (1967) Rheumatoid arthritis with IgA deficiency. *Amer. J. Dis. Child.* **113,** 41.

ISHIZAKA, T., ISHIZAKA, K., ORANGE, R.P. & AUSTEN, K.F. (1970) The capacity of human immunoglobulin E to mediate the release of histamine and slow reacting substance of anaphylaxis (SRS-A) from monkey lung. *J. Immunol.* **104,** 335.

JOHNSSON, S.G.O., HOGMAN, C.F. & KILLANDER, J. (1968) Quantitative immunoglobulin determination. Comparison of two methods. Estimation of normal levels and levels in persons lacking IgA and IgD. *Acta path. microbiol. scand.* **74,** 519.

KACAKI, J.N., BULLOCK, W.E. & VAUGHAN, J.H. (1969) Failure of lymphocyte transformation to gamma-globulins in rheumatoid arthritis. *Lancet,* **i,** 1289.

KENT, J.F. & FIFE, E.H. (1963) Precise standardization of reagents for complement fixation. *Amer. J. trop. Med.* **12,** 103.

LANSBURY, J. (1967) Methods for evaluating rheumatoid arthritis. *Arthritis and allied conditions* (Ed. by J. L. Hollander), p. 276. Lea & Febiger, Philadelphia.

LA POLLA, J.J. & THURMON, T.F. (1966) Lymphocyte transformation in autoimmune disorders: A preliminary study. *J. Pediat.* **69,** 1129.

LEVENTHAL, B.G., WALDORF, D.S. & TALAL, N. (1967) Impaired lymphocyte transformation and delayed hypersensitivity in Sjörgren's syndrome. *J. clin. Invest.* **46,** 1338.

LOCKSHIN, M.D. & ROUNSAVILLE, B. (1969) In vitro delayed hypersensitivity in rheumatic disease patients. *Arthr. and Rheum.* **12,** 677.

LOEWI, G. (1969) Immune inflammation in the synovium. *Proceedings of international symposium of immune complex disease* (Ed. by L. Bonomo & J.L. Turk), p. 82. Carlo Erba Foundation, Milan.

MANCINI, G., CARBONARA, A.O. & HEREMANS, J.F. (1965) Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry*, **2**, 235.

MESSNER, R.P., CAPERTON, E.M., KING, R.A. & WILLIAMS, R.C. (1969) Interactions among rheumatoid factors, G antibodies, lymphocytes, and phagocytes. *Ann. N.Y. Acad. Sci.* **168**, 93.

OUCHTERLONY, O. (1962) Diffusion-in-gel methods for immunological analysis. *Progr. Allergy*. **6**, 1.

PANUSH, R.S., BIANCO, N.E. & SCHUR, P.H. (1971) Serum and synovial fluid IgG, IgA, and IgM anti-gammaglobulin levels in rheumatoid arthritis. *Arthr. and Rheum.* (In press.)

PETERSON, R.D.A. & GOOD, R.A. (1968) Ataxia-telangiectasia. *Immunologic deficiency diseases in man* (Ed. by D. Bergsma), p. 370. National Foundation March of Dimes, New York.

PETTY, R.E., CASSIDY, J.T., BURT, A. & SULLIVAN, D.B. (1970) Plasma infusions in a patient with selective IgA deficiency and juvenile rheumatoid arthritis. *Arthr. and Rheum.* **13**, 344.

ROCKLIN, R.E., CHILGREN, R.A. HONG, R. & DAVID, J.R. (1970a) Transfer of cellular hypersensitivity in chronic mucocutaneous candidiasis *in vivo* and *in vitro*. *Cellular Immunology*, **1**, 290.

ROCKLIN, R.E., MEYERS, O.L. & DAVID, J.R. (1970b) An in vitro assay for cellular hypersensitivity in man. *J. Immunol.* **104**, 95.

ROCKLIN, R.E., REARDON, G., SHEFFER, A., CHURCHILL, W.H. & DAVID, J.R. (1970c) Dissociation between two in vitro correlates of delayed hypersensitivity: absence of migration inhibitory factor (MIF) in the presence of antigen-induced incorporation of 3H-thymidine. *Proceedings of the fifth leukocyte culture conference* (Ed. by J. Harris), p. 639. Academic Press, New York.

ROPES, M.W., BENNETT, G.H., COBB, S., JACOX, R. & JESSAR, R.H. (1959) Revision of diagnostic criteria for rheumatoid arthritis. *Arthr. and Rheum.* **2**, 16.

ROURKE, M.D. & ERNSTENE, A.C. (1930) A method for correcting the erythrocyte sedimentation for variations in the cell value percentage of blood. *J. clin. Invest.* **8**, 545.

SCHUR, P.H., BOREL, H., GELFAND, E.W., ALPER, C.A. & ROSEN, F.S. (1970) Selective gamma-G globulin deficiencies in patients with pyogenic infections. *New Engl. J. Med.* **283**, 631.

SHARP, J.T., CALKINS, E., COHEN, A.S., SCHUBART, A.F. & CALABRO, J.J. (1964) Observations on the clinical, chemical, and serological manifestation of rheumatoid arthritis, based on the course of 154 cases. *Medicine* (*Baltimore*), **43**, 41.

SUKERNICK, R., HANIN, A. & MOSOLOV, A. (1968) The reaction of blood lymphocytes from patients with rheumatoid arthritis against human connective tissue cells *in vitro*. *Clin. exp. Immunol.* **3**, 171.

TOMASI, T.B. (1968) Human immunoglobulin A. *New Engl. J. Med.* **279**, 1327.

WHALEY, K., GLEN, A.C.A., DICK, W.C., NUKI, G. & BUCHANAN, W.W. (1970) Delayed hypersensitivity in Sjögren's syndrome and rheumatoid arthritis. *Proceedings of the third symposium on impaired cell-mediated hypersensitivity in man* (Ed. by J. F. Jennings and D. J. Ward), p. 25, The Robert Jones and Agnes Hunt Orthopaedic Hospital Management Committee, Oswestry, England.

VYAS, G.N., HOLMDAHL, L., PERKINS, H.A. & FUDENBERG, H.H. (1969) Serologic specificity of human anti-IgA and its significance in transfusion. *Blood*, **34**, 573.