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Immunofluorescence studies for immunoglobulins and complement C3 in synovial joint membranes in psoriatic arthritis

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SUMMARY

Synovial tissues from fifteen patients with psoriatic arthritis were investigated with direct immunofluorescence staining for immunoglobulins (IgG, IgA and IgM) and from eight patients for complement component C3. As control groups, there were synovial tissues from seven patients with seropositive rheumatoid arthritis and five patients with meniscal tears. In psoriatic arthritis, immunoglobulins were found in plasma cells in 93% of the cases, always with the presence of IgG (93%) but also with IgA (47%) and IgM (7%). C3 could not be demonstrated. In seropositive rheumatoid arthritis IgG was demonstrated in all patients (100%), often together with IgA (43%) and IgM (57%). C3 was found in all of these patients. In patients with meniscal tears neither immunoglobulins nor C3 could be found.

The present findings indicate immunological activity in synovial joint membranes in psoriatic arthritis. The low amount of IgM and the lack of C3 suggest a difference compared to seropositive rheumatoid arthritis.

INTRODUCTION

In the pathogenesis of psoriasis, immunological processes may contribute essentially to the clinical picture, such as circulating autoantibodies against stratum corneum antigen (Krogh & Tønder, 1968), or antibodies against antigen in the nuclei of the basal cell layers of human epidermis (Cormane, 1976). Depression of circulating T lymphocytes causing the E-rosette phenomenon (Cormane, 1976; Gilhou *et al.*, 1976) and changes in the mitogen stimulation of lymphocytes (Gilhou *et al.*, 1976; Glinski & Jablonska, 1976) has been reported in psoriasis vulgaris.

In psoriatic arthritis (PSA), immunological studies on eluates from synovial joint tissues from three patients demonstrated the presence of IgG complexes (Munthe, 1970). In synovial tissues in rheumatoid arthritis (RA), immunoglobulins and complement have been found (Munthe & Natvig, 1970). Because of scant information on immunological mechanisms in PSA, synovial joint membranes from such patients were, in the present study, examined with the direct immunofluorescence technique for the presence of immunoglobulins and complement (C3).

MATERIALS AND METHODS

Tissues. Synovial joint membranes were obtained by synovectomy in bloodless fields by open surgery from fifteen patients with PSA (eight men and seven women). As control groups there were seven patients with a seropositive RA (one man and six women), and five men with meniscal tears. The diagnosis of PSA was based upon the presence of seronegative arthritis as judged by the Waaler–Rose and latex tests for rheumatoid factor (fifteen out of fifteen patients), mostly with an effect on distal interphalangeal joints (eleven out of fifteen patients). All of these cases demonstrated typical psoriasis of the skin at the time of the operation (fifteen out of fifteen patients), usually together with psoriatic changes of the nails (onycholysis, pitting) (eleven out of fifteen patients). In four out of eight cases with PSA examined, tissue-typing demonstrated the presence of the HLA-B27 antigen. The diagnosis of definite seropositive RA was based upon the ARA criterias (Bennett & Burch, 1967).

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Synovial membranes from patients with meniscal tears demonstrated no macroscopic changes. None of the patients in the control groups had psoriasis of the skin.

Methods. Immediately after synovectomy the synovial joint membranes were kept for a few minutes in 0·15 M NaCl at 4°C and subsequently quick-frozen in Tissue-Tec OCT compound (Lab-Tek Products Division, Miles Laboratories Inc., Naperville, Illinois, U.S.A.) using dry ice–acetone–isopentane and stored at −25°C until used.

Immunofluorescence studies. The following rabbit antisera labelled with fluorescein isothiocyanate (FITC) were used: anti-$F(ab')_2$, anti-IgG, anti-IgA, anti-IgM and anti-C3. FITC-labelled anti-C3 was obtained from Dakopatts A/S (Copenhagen, Denmark). The other antisera were produced in our laboratory by immunization with IgG (obtained from Kabi, Sweden), $F(ab')_2$IgG (obtained by pepsin digestion of IgG), IgA (isolated from a patient with myelomatosis by ion-exchange chromatography and gel filtration) and IgM (isolated from a patient with Waldenström's macroglobulinaemia by euglobulin precipitation and gel filtration). Antisera to the three Ig classes were absorbed in solution with $F(ab')_2$. All antisera were monospecific when tested in double-immunodiffusion analysis and immunoelectrophoresis against whole normal human serum or plasma.

IgG was isolated from the various anti-Ig antisera on DEAE–cellulose, concentrated to 7–10 mg/ml and dialysed against PBS. 10 μg of lyophilized FITC isomer I (Baltimore Biological Laboratories, Cockeysville, Maryland, U.S.A.) was added per mg of rabbit IgG. Unconjugated FITC was removed by gel filtration and fractions of antibody with optimal labelling were obtained by a slight modification of the method of Cebra & Goldstein (1965). The F/P ratios of the conjugates varied from 0·7–2·4 when measured as described by The & Feltkamp (1970). The final IgG concentrations used varied from 0·2–4·0 mg/ml. Direct immunofluorescence examinations were performed on 4 μm thick sections and with a Leitz Orthoplan microscope equipped for incident light. The conjugates were tested on selected tissues known to contain the various antigens. The specificity was further tested by blocking experiments performed with purified antigens, except for C3 which was not available. In all cases only the homologous protein inhibited the fluorescence.

RESULTS

Intracellular depositions of immunoglobulins

Psoriatic arthritis. Direct immunofluorescence studies (DIF) demonstrated intracellular depositions of immunoglobulins in the synovial joint membranes in fourteen out of fifteen (93%) of the patients with PSA (Table 1). The depositions were located in the cytoplasm of large mononuclear cells probably representing plasma cells (Fig. 1a). The cells were mostly localized in the synovial lining, sometimes reaching into the superficial cell layers. In some samples, plasma cells were few and often singularly

TABLE 1. Direct immunofluorescence studies on synovial membranes for the detection of immunoglobulins and complement in psoriatic arthritis and a control group of meniscal tears

			$F(ab')_2$		IgG		IgA		IgM		C3	
Tissue	Joint	HLA-B27	Ic*	Ec†	Ic	Ec	Ic	Ec	Ic	Ec	Ic	Ec
721	wrist		+		+		+		−			
910	knee		+		+		+		−			
1853	knee		+		+		+		−			
1964	MCP-I		−		−		−		−			
1973	wrist		+		+		−		−			
2329	knee		+		+		+		−			
2401	knee		+		+		+		−			
3363	DIP-II	+	+	+	+	+	−	−	−	−	−	−
3364	IP-I	+	+	+	+	+	−	−	−	−	−	−
3382	IP-I	+	+	+	+	+	−	−	−	−	−	−
3402	knee	−	+	+	+	+	−	−	−	−	−	−
3445	knee	+	+	+	+	+	+	+	−	−	−	−
3459	wrist	−	+	+	+	+	+	+	+	+	−	−
3475	MCP-I	−	+	+	+	+	−	−	−	−	−	−
3483	wrist	−	+	+	+	+	−	−	−	−	−	−
Control knees			−	−	−	−	−	−	−	−	−	−

DIP: Distal interphalangeal joint; MCP: metacarpo-phalangeal joint; IP: interphalangeal joint. (+) Positive result; (−) negative result.

* Intracellular deposition. † Extracellular deposition.

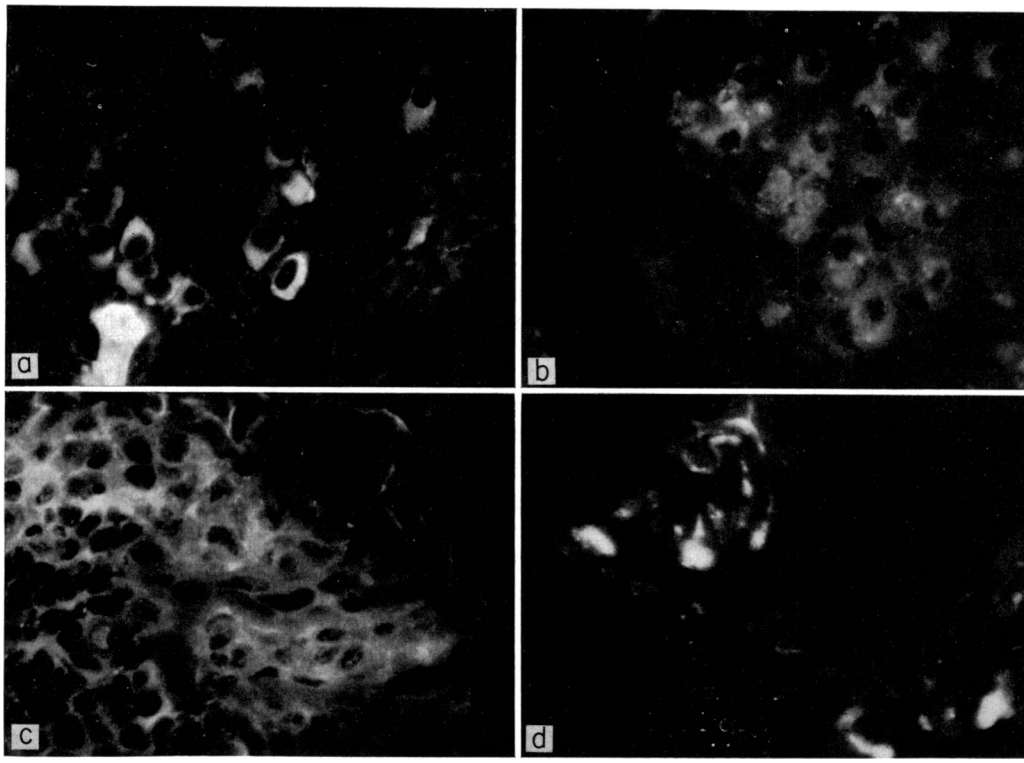

FIG. 1. Immunofluorescence micrographs from synovial joint membranes. Depositions of immunoglobulins in psoriatic arthritis: (a) $F(ab')_2$; (b) IgG; (c) IgG. Depositions of complement in seropositive rheumatoid arthritis: (d) C3. (Magnification × 530.)

distributed, in other sections they were numerous. Occasionally hundreds of plasma cells formed cell-clusters resembling 'germinal centers'. The cells were round or ovoid with large round excentrically situated nuclei. The ovoid cells were mostly situated in dense cell groups. The IF staining demonstrated either a homogeneous (Fig. 1a) or grainy pattern (Fig. 1b), and the degree of staining varied within the section. Regularly within the same cell group the same degree of staining was observed. The staining ranged from barely visible to very strong.

The major immunoglobulin was of the IgG class (Fig. 1b), always together with staining for $F(ab')_2$ (Fig. 1a) in fourteen out of fifteen cases (93%). IgA was found in seven out of fifteen cases (47%) (Fig. 1c) always together with IgG. In one case (7%) IgM was found together with IgG and IgA.

Seropositive rheumatoid arthritis. Generally, DIF studies demonstrated the same morphological picture as described in PSA. In all samples, in seven out of seven cases (100%), $F(ab')_2$ and IgG were found, in three out of seven cases (47%) together with IgA and in four out of seven cases (57%) together with IgM. In three out of seven cases (47%) IgG, IgA and IgM were found together in the same section (Table 2).

Meniscal tears. In this control group, immunoglobulins could not be demonstrated.

Extracellular depositions of immunoglobulins

Only synovial membranes which could be examined a few days after an operation were used for investigation of extracellular immunoglobulins, to avoid the unspecific background staining of the older tissue samples.

Psoriatic arthritis. In eight out of eight (100%) of the synovial membranes (Table 1) investigated, extracellular depositions of immunoglobulins were found. In all cases, IgG and $F(ab')_2$ were demonstarted, in two out of eight cases (25%) together with IgA. In one patient (one out of eight—12·5%)

TABLE 2. Direct immunofluorescence studies in synovial membranes from patients with seropositive rheumatoid arthritis for the detection of immunoglobulins and complement

Tissue No.	$F(ab')_2$		IgG		IgA		IgM		C3	
	Ic*	Ec†	Ic	Ec	Ic	Ec	Ic	Ec	Ic	Ec
3341	+	+	+	+	+	+	+	+	+	+
3387	+	+	+	+	+	+	+	+	−	+
3392	+	+	+	+	+	+	+	+	−	+
3501	+	+	+	+	−	−	+	+	−	+
3517	+	+	+	+	−	−	−	−	+	+
3519	+	+	+	+	−	−	−	−	+	+
3529	+	+	+	+	−	−	−	+	+	+

(+) Positive result; (−) negative result.
* Intracellular deposition. † Extracellular deposition.

IgG, IgA and IgM were found together in the same section with the dominating presence of IgG and IgM. The depositions demonstrated a grainy or cloudy pattern, often located in the region with or without intracellular immunoglobulins. The deposits were located in the synovial membranes, often reaching into the superficial cell layers.

Seropositive rheumatoid arthritis. The pattern of the extracellular depositions of immunoglobulins were generally the same in this group as in the group of PSA. In one patient, IgM was only present extracellularly, otherwise the immunoglobulins were always present extra- and intra-cellularly in the same samples (Table 2).

Mensical tears. No immunoglobulins could be demonstrated.

Complement C3

Only tissue samples investigated a few days after an operation were used for the study of depositions of complement C3.

Psoriatic arthritis. DIF studies in samples from eight patients with PSA could not demonstrate the presence of complement C3.

Seropositive rheumatoid arthritis. Extracellular depositions of complement C3 were demonstrated in all samples from patients in this group (seven out of seven). Intracellular depositions were found in four out of seven patients. All these depositions were rather faint (Fig. 1d). Small localized areas of the sections showed extracellular accumulations of spotted IF, which in one sample was more pronounced. Intracellular depositions were mostly observed in areas with round cells containing immunoglobulins (three out of seven patients) or in synovial cells lining the joint cavity (two out of seven patients).

Meniscal tears. In this control group, no complement C3 could be found.

DISCUSSION

The classification of PSA is difficult, mainly because of the lack of a universally accepted definition (Farber *et al.*, 1974; Wright & Moll, 1971). However, epidemiological, radiographical and laboratory investigations now characterize PSA as a disease which is generally considered to represent a clinical entity (Sherman, 1952; Wright, 1956; Wright & Moll, 1973; Theiss *et al.*, 1969). This view is supported by recent results of tissue-typing demonstrating a significant increase of HLA-B27 in PSA, especially in cases with spondylitis and sacroiliitis (Brewerton, 1976; Lambert *et al.*, 1976). According to clinical experience a connection seems to exist between the activity of the arthritis and the cutaneous changes.

This relation is best demonstrated in the mutilating form of psoriatic arthritis which is regularly accompanied by aggressive cutaneous changes, often as exfoliating erythrodermia.

Currently, the definition of PSA as a disease with 'psoriasis of the skin with seronegative arthritis' (Wright & Moll, 1971), together with other characteristic features like distal interphalangeal arthritis, nail changes and possibly with the presence of the HLA-B27 antigen seems most adequate. As indicated by Holzmann *et al.* (1967) using sensitive scintographic examinations with radionuclides, subclinical inflammation of the joints in patients with psoriasis vulgaris may be more frequent than is generally assumed.

Routine light microscopical examinations, from the same synovial tissue as investigated in this study, all demonstrate discrete inflammatory changes in the synovial membranes with the presence of small lymphocytes and plasma cells. In PSA, and in the control groups of seropositive rheumatoid arthritis and meniscal tears, direct IF studies also revealed varying amounts of fibrinogen–fibrin-related antigen (FR-antigen) at the surface of the membranes against the joint cavity and intracellularly in the superficial synovial cell layers (Fyrand & Mellbye, 1977). In rheumatoid arthritis similar observations have been reported by others (Bach-Andersen & Gormsen, 1970).

In 1970, Munthe demonstrated high amounts of IgG complexes in eluates from synovial tissue from three patients with PSA. In one of these cases free IgM rheumatoid factor was found. In rheumatoid arthritis, synovial tissue showed the presence of plasma cells with intracellular depositions of IgG, IgM and IgA, together with complement. IgG was the dominating immunoglobulin. IgM was mostly present in seropositive and missing in seronegative rhematoid arthritis (Munthe & Natvig, 1970).

In our study, the presence of complement C3 could not be demonstrated in eight patients with PSA. This is in contrast to the findings in the group of seven patients with seropositive rheumatoid arthritis, where complement C3 was found in all cases. This might be due to the presence of the subclass IgG4 known not to activate complement by the classical pathway. However, the unique presence of IgG as IgG4 in all sections of PSA is unlikely. Possibly the lack of C3 in PSA reflects a different immunological mechanism in relation to seropositive RA. The presence of IgM was also different in the two groups. In PSA, one out of eight (13%) of the patients demonstrated IgM in contrast to four out of seven (57%) in the group of rheumatoid arthritis.

The significance of the presence of immunoglobulins in synovial joint membranes in PSA is unknown. It may suggest an immunological background in the development of the arthritis. The possibility of a co-existing relation between the immunological findings reported in psoriasis vulgaris (Cormane *et al.*, 1976; Glinski & Jablonska, 1976; Gilhou *et al.*, 1976) and the findings reported in this study must be considered. Studies on rheumatoid arthritis have demonstrated that a local *in vitro* production was found in synovial tissue (Smiley *et al.*, 1968), and in a clinical study 12–24% of the IgG present in the synovial fluid in rheumatoid arthritis was produced locally (Zvaifler, 1971). This may also be the case in PSA. This hypothesis is supported by a study demonstrating a higher concentration of IgG in synovial fluid compared with the corresponding serum in patients with PSA (Hedberg, 1963). Tapanes, Rawson & Hollander (1972) demonstrated increased amounts of serum anti-Ig of IgG and IgA classes in seropositive, seronegative and juvenile rheumatoid arthritis. In PSA, the anti-IgG concentrations were within normal range. They proposed this difference to be used as a diagnostic aid in the differentiation of PSA from other types of rheumatoid arthritis. The lack of complement C3 in PSA in our study indicates another difference compared to seropositive rheumatoid arthritis. This difference may offer one explanation of the generally more benign development of the arthritis in psoriatics.

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