LOCALISED IMMUNE COMPLEXES AND SLIPPED UPPER FEMORAL EPIPHYSIS

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Slipped upper femoral epiphysis remains a disease of unknown aetiology. Recent evidence has bolstered speculation that the immune system may play a role in the aetiology or pathogenesis of slipped epiphysis or of one of its complications, chondrolysis. This study reports the finding of immune complexes in the synovial fluid of all but one hip affected with slipped epiphysis in a consecutive series. In seven patients, immune complexes were detected by both the Raji cell assay and C1q-binding assay; in two, by the C1q-assay only; and in one, by the Raji cell assay only. No patients had immune complexes in the serum. Twenty-one patients with synovitis of the knee or hip caused by a variety of disorders served as the control group. Two of these patients had immune complexes in their synovial fluid. It appears that the immune complexes characterise the synovitis found with slipped upper femoral epiphysis as distinct from most other synovitides.

Slipped upper femoral epiphysis remains a disease of unknown aetiology despite abundant investigation and speculation. Other than its being limited to the proximal femoral epiphysis, perhaps its most unusual feature is that occurrence is limited to the narrow age range of puberty. This propensity, during a period of hormonal change affecting skeletal development, has led to speculation that this disease may be secondary to hormonal imbalance. Despite some laboratory evidence (Harris 1950; Hillman, Hunter and Barrow 1957; Bright, Burstein and Elmore 1974) and the occasional association with an endocrine abnormality (Chiroff, Sears and Slaughter 1974; Fidler and Brook 1974; Heatley, Greenwood and Boase 1976; Moorefield *et al.* 1976), no such abnormalities are apparent in the majority of cases.

Trauma should also be considered in the aetiology. Structural changes at the time of puberty may alter the strength of the epiphysal plate in animals (Bright *et al.* 1974) and in man (Chung, Batterman and Brighton 1976). In addition, epiphysal fractures in children have a peak age incidence similar to that of slipped epiphysis (Peterson and Peterson 1972). However, there are three factors that make it difficult to accept the concept that

slipped upper femoral epiphysis is an accident like an epiphysal fracture of the distal radius.

The first is that children with slipped upper femoral epiphysis do not experience the degree of trauma needed to fracture such a large epiphysal plate, nor do other large epiphyses show similar slow slipping. Secondly, a familial occurrence has been demonstrated (Wilson, Jacobs and Schecter 1965; Rennie 1967, 1982); this may implicate genetic factors, but the incidence varies in different parts of the United States (Kelsey, Keggi and Southwick 1970), so that the environment also may be a factor.

The third and most important indication that it is a chronic disease unrelated to trauma is its constant association with synovitis even before slipping can be demonstrated. Although this has been noted for years (Howorth 1949), it has been given little attention. Recent investigation showing abnormalities of immune regulation associated with slipped femoral epiphysis reawakened interest in a possible immunological mechanism causing the synovitis and its possible role in slipped epiphysis and in chondrolysis (Mankin *et al.* 1975; Eisenstein and Rothschild 1976). Such studies suggest a hyper-reactive state such as that seen with infectious processes or abnormalities of host defence.

Immune complexes formed by the reaction of antigen and antibody are a part of the normal homeostatic mechanism by which the host neutralises and eliminates foreign antigen. In certain circumstances, however, the immune complexes may be harmful to the host and cause disease. Because in previous work we found immune complexes in the synovium of some patients with slipped femoral epiphysis (Morrissey, Kalderson and Gerdes 1981), we undertook this study to seek more direct evidence for their presence and role.

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0301-620X/83/5122 \$2.00

MATERIALS AND METHODS

Twelve consecutive patients undergoing treatment for slipped epiphysis were studied. Diagnostic criteria included the characteristic clinical findings with radiographic evidence of displacement of the proximal femoral epiphysis. Except for one patient with hypothyroidism who was under treatment, no patient had any discernible cause for the slipped epiphysis.

Specimens of serum were obtained. Then a modified Watson-Jones incision was made. Before incising the capsule the hip joint was aspirated to obtain a sample of synovial fluid; the capsule was then incised and a specimen of synovium removed for biopsy. All specimens were immediately frozen at -70 degrees Celsius.

An additional group of patients with diseases of the hip or knee associated with synovitis who underwent arthroscopy or operation were studied in a similar manner.

Immunochemical assays. Total haemolytic complement (CH_{100}) was measured by standard radial diffusion techniques, using Quantiplates (Kallested Laboratories). The complement components C3 (supplied by Hyland Laboratories, Los Angeles, California) and C4 (supplied by Behring Diagnostics, Sommerville, New Jersey) were used. Complement measurements were made by radial immunodiffusion, and the immunoglobulins, IgG, IgA and IgM, by laser nephelometry. These assays were performed on both synovial fluid and serum.

Detection of immune complexes. Synovial fluid and serum were examined for the presence of immune complexes by the Raji cell indirect fluorescent method and by the C1q solid-phase assay. The Raji cell lines were derived from a patient with Burkitt's lymphoma and provided by Mr M. Vincent, University of Arkansas for Medical Sciences. Cells were cultured in Eagle's minimal essential medium and concentrated to 5×10^7 per millilitre. To diminish background fluorescence caused by 7S IgG binding to receptors for IgG Fc, Raji cells were first incubated with human IgG, washed, and reacted with Fab rabbit antihuman IgG (Theofilopoulos *et al.* 1974). After incubation with test serum or synovial fluid, fluorescein isothiocyanate-labelled rabbit antihuman IgG serum was added and qualitative assessment of fluorescence-positive cells was accomplished. Negative and positive control specimens were included and all preparations coded before being evaluated by two experienced microscopists. Fluorescence was rated as follows: 0 none or equivalent to background, + unequivocal, ++ brilliant.

The C1q-binding assay was performed by standard methods (Hay, Nineham and Roitt 1976). Human C1q was separated from serum, radio-iodinated by the lactoperoxidase method (Heusser *et al.* 1973) to a specific activity of 1.25 to 2.0 microcuries of iodine-125 per microgram and stored at -70 degrees Celsius. The standard for comparison was 100 per cent precipitation by 20 per cent trichloroacetic acid, and patient samples were recorded as per cent activity against this standard. Normal negative serum controls for this assay were less than 15 per cent. All positive fluids were screened for hepatitis B antigen and rheumatoid factor by standard techniques.

Immune complexes in four positive samples of synovial fluid were further characterised as to molecular size by ultracentrifugation at 170 000 *g* for 18 hours in sucrose gradients. Density gradients were prepared either in Veronal-buffered saline (pH 7.3) or in acetate buffer (pH 4.0) and centrifugation was accomplished in a Beckman Model L ultracentrifuge with a swinging SW 50 L rotor. Fractions of synovial fluid layered over gradients were collected with a fractionator (Instrumentation Specialties Company Inc., Lincoln, Nebraska). Human albumin, IgG and IgM served as the 5S, 7S and 19S markers. After fractionation, samples were tested by C1q-binding assay.

To test complexes for DNA and anti-DNA antibodies, positive synovial fluid was also incubated with DNAase using standard methods (Zubler, Nydegger *et al.* 1976).

Immunopathological studies. Biopsies of synovium were studied by light microscopy and by direct immunofluorescence. Cryostat sections were washed, fixed in ether-ethyl alcohol, and stained with heavy-chain specific goat antihuman IgG, IgA and IgM (Melyo Laboratories) and C3 (Hyland Laboratories).

RESULTS

There were 12 patients in this study (Table I); eight were black and seven were boys. Five slips were rated as mild, seven as moderate, and none as severe. One acute slip occurred in this series. All but one of these patients were followed for at least one year and no chondrolysis or avascular necrosis had developed.

The control group comprised 21 patients (Table II) with diseases that were characterised by synovitis and required surgical treatment or arthroscopy, thus affording the opportunity to obtain appropriate specimens.

Of the 12 patients with slipped epiphysis, synovial fluid was obtained in 11. In the other patient, abundant synovial fluid was present but could not be withdrawn before capsulotomy. Immune complexes were detected in 10 of the 11 samples of synovial fluid. In seven patients they were detected by both assays; in two by the C1q-binding assay only, and in one by the Raji cell assay only. Hepatitis B antigen and rheumatoid factor were not found in any of these positive specimens. No immune complexes were detected in the sera of these patients.

Four samples of synovial fluid which were positive in the C1q-binding assay were examined after ultracentrifugal analysis. After fractionation at neutral pH, C1q reactivity was present in fractions corresponding to the 12–16S area of the gradients. C1q-binding was greatly reduced in fractions obtained from acid gradients. Treatment with DNAase did not produce appreciable reduction in the C1q-binding activity of synovial fluids. The immune globulin and complement levels in the sera of these patients were normal.

Normal values for synovial fluid were not available from our laboratory, but results for immunoglobulins and complement in the patients with slipped epiphysis were comparable with those of the control groups. Our conclusion is that there was no significant alteration of the serum or synovial fluid levels of immunoglobulins or complement. Immune complexes were detected in the synovial fluid of only two patients in the control group. One of these was a six-year-old black boy with known juvenile rheumatoid arthritis: the synovial fluid aspirated from his knee was positive on the Raji cell assay, but not in the C1q-binding assay. The other patient was an eight-year-old white boy with a low lumbar myelomeningocele and a painful subluxated hip in which a marked synovitis was observed at operation; the synovial fluid was positive on both the Raji cell assay and the C1q assay. No control patients had immune complexes detected in their sera.

No patient in either group demonstrated positive synovial immunofluorescence, not even the control patient with juvenile rheumatoid arthritis. In the patients with slipped epiphysis the synovial biopsies showed oedema, proliferation of synovial cells, villi formation, increased vascularity, and infiltrate of lymphocytes and plasma cells. The plasma cells were most notable in the patients with slipped epiphysis, often appearing in large groups.

Table I. Immunological details of 12 patients with slipped upper femoral epiphysis

Case	Age (years)	Race	Sex	Synovial fluid (mg/dl)							Serum (mg/dl)									
				Raji	Clq (%)	C3	C4	IgG	IgA	IgM	CH ₁₀₀	Raji	Clq (%)	C3	C4	IgG	IgA	IgM	CH ₁₀₀	
1	10	W	F	+	54	54	14	666	70	35	26	-	-	175	39	1142	187	112	70	
2	10	B	F	+	20	57	16	376	38	28	35	-	-	334	59	890	162	89	155	
3	15	B	M	+	23	37	12	279	35	23	32	-	-	168	48	994	146	123	92	
4	11	B	F	+	6	40	8	551	24	83	26	-	-	212	35	968	84	292	55	
5	13	B	M	+	61	31	12	617	42	39	35	-	-	156	52	1125	171	90	76	
6	16	W	M	+	37	38	8	534	22	75	29	-	-	129	36	974	74	298	57	
7	14	B	M	-	8	37	12	379	35	23	32	-	-	133	31	862	181	67	66	
8	12	B	M	No analysis made										-	220	32	1148	168	148	85
9	12	W	F	+	52	38	9	407	83	47	41	-	-	205	35	1057	171	65	90	
10	12	W	F	+	19	39	18	528	37	34	37	-	-	192	71	1029	223	101	180	
11	13	B	M	-	45	33	12	430	61	52	30	-	-	131	52	943	95	130	67	
12	14	B	M	-	18	42	9	410	28	25	31	-	-	142	46	950	123	82	71	

Table II. Immunological details of 21 control patients

Case	Age (years)	Race	Sex	Synovial fluid (mg/dl)						Serum (mg/dl)						Diagnosis				
				Raji	Clq (%)	C3	C4	IgG	IgA	IgM	CH ₁₀₀	Raji	Clq (%)	C3	C4		IgG	IgA	IgM	CH ₁₀₀
13	10	W	F	-	-	40	12	385	29	33	38	-	-	216	61	917	134	95	123	Spina bifida; Charcot knee
14	13	W	F	-	-	34	9	526	30	41	37	-	-	160	52	1248	167	99	116	Synovial chondromatosis
15	12	W	F	-	-	27	11	408	16	25	42	-	-	225	37	1173	149	122	94	Intra-articular fracture (seven days earlier)
16	12	B	F	-	-	22	6	737	55	64	20	-	-	184	56	1239	120	148	83	Gonococcal arthritis
17	2	W	F	-	-	63	15	389	38	41	36	-	-	169	25	717	67	180	38	Congenital dislocation of the hip
18	3	W	F	-	-	52	12	390	14	57	35	-	-	176	18	801	30	173	76	Congenital dislocation of the hip
19	2½	W	F	-	-	40	10	256	27	35	19	-	-	183	32	759	84	165	106	Congenital dislocation of the hip
20	4	W	F	-	-	26	13	315	30	24	33	-	-	164	49	915	68	123	87	Juvenile rheumatoid arthritis
21	6	B	M	+	-	38	14	301	33	19	37	-	-	139	52	631	40	58	85	Juvenile rheumatoid arthritis
22	11	W	M	-	-	28	8	417	15	29	23	-	-	175	40	1157	119	150	99	Osteoid osteoma of the femoral neck
23	13	B	M	-	-	40	8	376	24	38	36	-	-	126	45	928	97	145	87	Torn meniscus
24	16	B	F	-	-	31	9	483	36	35	44	-	-	213	42	1416	135	166	110	Torn meniscus
25	6	W	M	-	-	45	4	609	101	61	20	-	-	175	50	1252	191	174	76	Septic knee
26	13	B	F	-	-	46	12	392	51	40	55	-	-	133	22	842	184	212	63	Chondromalacia patellae
27	14	B	M	-	-	68	18	565	37	42	34	-	-	139	33	1441	93	187	55	Chondromalacia patellae
28	9	W	M	-	-	70	14	472	95	28	50	-	-	127	38	789	80	123	74	Perthes' disease
29	15	W	M	-	-	<10	3	293	17	35	11	-	-	176	47	972	136	111	98	Retropatellar pain, but no chondromalacia
30	16	W	M	-	-	52	8	415	34	42	18	-	-	164	17	1361	178	120	92	Chondromalacia
31	11	B	M	-	-	44	10	510	40	37	55	-	-	124	26	1097	224	170	55	Sickle-cell disease; knee effusion
32	7	W	M	-	-	49	10	176	20	16	22	-	-	101	38	596	48	46	105	Septic knee
33	8	W	M	+	43	31	8	538	49	57	0	-	-	141	20	1008	104	188	43	Spina bifida; painful subluxated hip

DISCUSSION

Due largely to improved methods of detection, immune complexes have now been demonstrated in a number of diseases (for example infectious, gastro-intestinal, neurological and dermatological disorders), in which an alteration of immune function was not previously suspected (Zubler, Lange *et al.* 1976; Lambert *et al.* 1978). Except for the rheumatoid diseases, disordered immune function has not been implicated as an aetiological factor in orthopaedic diseases.

Immune complexes are formed by the reaction of antibody with antigens which are generated from micro-organisms, autologous cells or any exogenous substance. Disease does not always result since formation of complexes is a normal mechanism by which the host neutralises and eliminates foreign antigen. If antigen does persist, as is most commonly recognised with some infectious and auto-immune diseases, complexes may then trigger a series of biological events which could prove detrimental to the host. These events involve fixation of complement, release of chemotactic factors and vaso-active amines, increased vascular permeability, and infiltration with polymorphonuclear and mononuclear leucocytes. The result is tissue destruction.

There are two problems with this present study which are common to any similar study that seeks the presence of immune complexes: is the material identified truly an immune complex and what, if any, role does it play in the disease process?

There currently exist over 20 indirect methods to detect immune complexes, each based on differing properties of the various complexes. While there is no question of the ability of these various tests to detect the complexes, there is also no question that they can detect more.

The two methods chosen for this study, C1q and Raji cell assay, are acknowledged to be two of the best methods to detect immune complexes (Lambert *et al.* 1978). Although both are IgG specific, they have significant differences. The Raji cell assay is based on the fact that these cells do not have any membrane-bound immunoglobulins, but have receptors for complement-bound protein. Some of these binding sites may first be blocked, thus diminishing the chances that something other than complement will be detected. The cells are then used to detect immune complexes bound to complement. The C1q-binding assay relies on the fixation of C1q, the first component of complement, to the specific receptor of the complexed immunoglobulin. The method by which this assay was performed in this study (Hay *et al.* 1976) significantly reduces binding of substances other than immune complexes. By the methods described, immune complexes were found in the synovial fluid from 10 of 11 patients with slipped femoral epiphysis.

To strengthen further the presumption that the substances detected were immune complexes, all positive sera were screened for rheumatoid factor and hepatitis B

antigen, two substances which are known also to result in a positive C1q assay. None were found. Furthermore, DNAase did not alter the C1q-binding assay, thus eliminating DNA and anti-DNA antibody as a major portion of the complexed material. In addition, material identified on C1q binding in four patients was found to sediment by ultracentrifugation in the 12–16S range on sucrose density gradients, a finding compatible with immune complexes. This material demonstrated that the ability to bind C1q was lost after acid dissociation at pH 4.0.

Previous work in our laboratory has demonstrated the presence of IgG and C3 in the synovium of three of 15 consecutive patients with slipped epiphysis (Morrissey *et al.* 1981). Two of these patients subsequently developed chondrolysis. The presence of IgG and C3 in the same tissue is presumptive evidence for the presence of immune complexes (Fudenberg *et al.* 1976; Hamashima 1976). Efforts to detect immunoglobulins, and presumably immune complexes, in synovial tissue by immunofluorescent staining were negative in these patients, which may be explained, in part, by the fact that two of these three patients developed chondrolysis while no patients in this present study did so. The histological appearance of the synovitis with plasma cell and lymphocyte infiltration is certainly compatible with an immune reaction.

Direct evidence that the immune complexes are participating in the pathological process is not available from this study. The fact that immune complexes can be isolated from joints affected by disease does not prove that the immune complexes are aetiological factors. Nevertheless, their identification in synovium and synovial fluid has been accepted as evidence for their participation in rheumatoid arthritis. In the current study, immune complexes were isolated from the joints of 10 patients with symptomatic slipped upper femoral epiphysis. Except for two control patients, immune complexes were not found at the site of the disease process in a variety of other orthopaedic disorders associated with synovitis. This finding of immune complexes at the site of a particular disease and their absence from a variety of other diseases that affect the same tissues and organs is strongly suggestive that they play a part in the pathological process.

Although values for immunoglobulin and complement levels in synovial fluid are difficult to obtain, we did not observe any significant alteration from the control group or within the study group. These findings most likely represent a low level of immunoglobulin consumption and a concomitant low level of immune complex formation in the joint. It needs to be emphasised that these measurements reflect only one particular time in the disease process of each patient. In the present series, circulating (serum) immune complexes were not observed, and levels of IgG, IgA, IgM, C3, C4 and CH₁₀₀ were comparable to normal values and to those of control study patients. These results conflict with those in

previous reports (Mankin *et al.* 1975; Eisenstein and Rothschild 1976), but differences in patient populations may account for these discrepancies. None of the patients in this series has yet had chondrolysis. In addition, alterations of polyclonal serum immunoglobulins are an insensitive index of immune system activity. Our data suggest that immune complex activity is localised to specific joints, at least at the time of clinical presentation with slipped epiphysis.

The absence of circulating immune complexes in these patients differs from the frequent findings of such complexes in sera from patients with rheumatoid arthritis;

rheumatoid arthritis, however, is the exception. Most other inflammatory arthritides are associated with immune complexes, which are found in the joints but rarely in the serum (Zubler, Nydegger *et al.* 1976; Hardin, Steere and Malawista 1979).

The importance of this study is to differentiate the synovitis seen in slipped upper femoral epiphysis from that seen in most other orthopaedic afflictions: it is different by virtue of the presence of immune complexes in the synovial fluid. Such findings point to a potentially rewarding new line of investigation into this peculiar disease entity.

This work was supported by a grant from the Orthopaedic Research and Education Foundation.

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