Immunohistochemical demonstration of CD23 expression on lymphocytes in rheumatoid synovitis

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Abstract
The leucocyte antigen CD23 is expressed by B lymphocytes following activation by a number of stimuli and functions as an IgE receptor, and in its soluble form, as a putative B cell growth factor. The expression of CD23 on the surface of lymphocytes in paraffin wax sections of synovial biopsy specimens was studied using a novel mouse monoclonal antibody, BU38. Specimens were investigated from nine cases of rheumatoid arthritis, six cases of osteoarthritis, and eight cases of chronic inflammation in articular and non-articular tissues. CD23 was expressed on a high proportion of lymphocytes in all forms of chronic inflammation and was not specific for rheumatoid arthritis. It may be a characteristic feature of any chronic inflammatory response. As CD23 was found on the surface of lymphocytes in many cases of these arthritides, sCD23 in serum or synovial fluid may yet prove a useful marker for the severity of the inflammatory infiltrate.

Rheumatoid arthritis is a chronic inflammatory disease which predominantly affects synovial tissues, resulting in irreversible articular damage and loss of function. Most patients with rheumatoid arthritis have serum rheumatoid factors consisting of immunoglobulins with specificity for the Fc fragment of IgG. Synovial lymphocytes have the capacity to produce rheumatoid factors, and the rheumatoid synovium is infiltrated with activated B and T cells.[1 2] A lymphocytic infiltrate is also seen, however, in other chronic synovitides.[3 4] Although the aetiology of rheumatoid arthritis is unknown, there is an association with HLA DR4,[5 6] and a defect in cellular immunity with abnormal production of and response to cytokines has been postulated.[7 8] Certain infectious agents including Epstein Barr virus (EBV) have also been implicated in the pathogenesis of the disease.[9–11]

The low affinity IgE receptor (CD23) is a 45 kilodalton molecule expressed on IgM/IgD bearing B lymphocytes following activation,[12 13] as well as on a number of other cell types including follicular dendritic cells and Langerhans' cells.[12 14–16] CD23 expression on B lymphocytes is particularly increased following infection by EBV,[17] but also occurs after activation by a number of other stimuli such as interleukin 4 and so is not specific to EBV infection.[12] CD23 also exists in a 25–30 kilodalton soluble form (sCD23) that is secreted or can be formed by cleavage of surface CD23.[18] This sCD23 has been suggested to have autocrine B lymphocyte growth factor activity,[19 20] although gene cloned sCD23 does not have this property.[21]

In this study we investigated the expression of CD23 by lymphocytes in paraffin wax embedded synovial biopsy specimens from patients with rheumatoid arthritis and osteoarthritis. Several specimens of other tissues showing chronic inflammation were also studied. A novel mouse monoclonal antibody, BU38, with the particular advantage of being able to visualise CD23 in paraffin wax sections[22] was used.

Methods
Archival paraffin wax embedded synovial biopsy specimens were obtained from the Department of Histopathology, East Birmingham Hospital. All sections of each specimen were reviewed, and those showing changes of chronic inflammation with a prominent chronic inflammatory cell infiltrate were chosen for further study. These included nine cases of rheumatoid arthritis, six of osteoarthritis, and three cases of non-specific synovial inflammation. The clinical case notes of all these patients were reviewed to confirm these diagnoses. Particular note was made of the rheumatoid factor concentration, radiological changes, the presence of morning stiffness, haemoglobin concentration and erythrocyte sedimentation rate (ESR) as indices of disease activity at the time of the biopsy.

Five biopsy specimens showing chronic inflammation in other tissues were also chosen for study. These consisted of non-specific dermatitis, chronic cholecystitis, branchial cyst, Crohn's disease of the ileum and chronic gastritis.

All the specimens had been fixed for at least 18 hours in 10% formol-saline and then processed as for routine histological examination. Sections were cut at 3 μm thickness, attached to glass slides, and incubated at 40°C for 18 hours and then dewaxed and

Table 1   Summary of clinical data and investigations performed in conjunction with synovial biopsy

Case No	Age (y)	Sex (M/F)	Morning stiffness	RF concentration		Hb (g%)	ESR (mm/h)	x ray	Biopsy site
				Lx	R-W				
Rheumatoid arthritis:									
1	61	F	+	+	+	12·7	105	RA	R Knee
2	73	F	+	+	+	11·9	ND	RA	L Knee
3	38	F	+	+	+	12·3	15	RA	R Wrist
4	57	M	+	+	−	13·7	ND	RA	L Wrist
5	77	M	+	+	−	10·7	118	RA	R Elbow
6	46	F	+	+	+	13·5	ND	RA	R Wrist
7	35	M	+	+	−	13·3	37	NAD	R Knee
8	56	F	+	+	+	13·2	28	RA	R Hand
9	34	F	+	+	+	13·1	25	RA	L Knee
Osteoarthritis:									
10	43	F	−	ND	ND	13·2	ND	OA	R Hip
11	77	F	−	ND	ND	12·5	ND	OA	R Hip
12	77	F	−	−	−	13·8	24	OA	L Hip
13	50	F	−	−	−	14·3	8	OA	L Hip
14	62	F	−	ND	ND	12·7	ND	OA	R Knee
15	68	F	− ·	ND	ND	12·3	6	OA	R Hip
Non-specific joint inflammation:									
16	12	F	−	−	−	12·5	9	NAD	R Ankle
17	18	F	+	−	ND	13·7	15	NAD	R Knee
18	28	M	−	−	−	15·5	5	NAD	R Ankle

RF = rheumatoid factor; Lx = Latex agglutination test; R-W = Rose-Waller test; Hb = blood haemoglobin concentration in grams per decilitre; ESR = erythrocyte sedimentation rate in millimetres per hour; ND = not done; RA = diagnostic or consistent with rheumatoid arthritis, OA = diagnostic or consistent with osteoarthritis; NAD = nothing abnormal discovered.

rehydrated. After the addition of trypsin, and blocking non-specific antibody binding with normal swine serum at a dilution of 1 in 20, the BU38 monoclonal antibody (The Binding Site Ltd, Birmingham) was applied to the sections at a dilution of 1 in 100. Control sections of the same specimens were stained with another ascitic fluid antibody, BU31, which recognises an antigen associated with nuclear envelopes. A standard alkaline phosphatase anti-alkaline phosphatase (APAAP) detection system was used. The bridging antibody used was a rabbit anti-mouse immunoglobulin reagent (Dakopatts; No Z259) at a dilution of 1 in 50. The APAAP complex (Dakopatts; No D651) was used at a dilution of 1 in 100. A second cycle of bridging antibody and APAAP complex was performed. All antibody incubations were performed for 30 minutes. The chromogen was prepared freshly before use by dissolving 25 mg naphthol AS-BI phosphate (Sigma; No N2250) in 1 ml dimethylformamide, adding this to 50 mg levamisole (Sigma; No L9756) in 50 ml 0·05 M TRIS buffer (pH 8·7), and then adding 50 mg Fast Red TR salt (Sigma; No F1500). The sections were incubated in this solution for 20 minutes at room temperature, washed, counterstained in Mayer's haematoxylin and then mounted in an aqueous medium.

All the sections were examined by two pathologists (EH and DCR) who assessed the degree of positivity (negative, weak, moderate or strong) and the percentage of lymphoid cells stained (none, less than 50%, more than 50% but less than 90% and more than 90%).

Results

Table 1 gives a summary of the clinical data concerning all the patients with joint disease included in this study. All of the patients who had been classified as having rheumatoid arthritis on clinical grounds were positive for rheumatoid factor, and all but one had radiological changes consistent with rheumatoid arthritis.

The results of the immunohistochemical staining on all of the specimens investigated is summarised in table 2. CD23 expression was moderately or strongly positive in 50% or more lymphocytes in six of the nine cases of rheumatoid arthritis. Two of the cases of rheumatoid arthritis, however, were negative for CD23, and in the remaining case only a small proportion of the lymphocytes in the inflammatory infiltrate were positive (figure). There was no apparent relation between CD23 positivity and age, sex, or disease activity, as measured by haemoglobin concentration and ESR at the time of the biopsy.

In the group of six biopsy specimens from patients with osteoarthritis one showed no CD23 staining, one had weak staining, two showed moderate staining and two showed strong staining in more than 50% of the lymphocytes. There was also a spectrum of weak to strong CD23 positivity in the three synovial biopsy specimens considered to show non-specific inflammation. There was moderate or strong CD23 positivity of more than 50% in all of the specimens showing chronic inflammation in non-articular tissues. None of these patients had any evidence of joint disease.

In both synovial and non-articular tissues that had pronounced CD23 positivity in lymphocytes, there was CD23 positivity in endothelial cells of small vessels within the region of the inflammation. Vessels away from areas of lymphoid infiltrate and in biopsy specimens with no or only weak CD23 positivity did not show this CD23 staining.

Discussion

Recent research has focused on the role of cytokines in rheumatoid arthritis, because these molecules may be responsible for the recruitment of cells and their activation within

Table 2 Assessment of CD23 positivity in lymphocytes from each of the biopsy specimens studied

Case No	Nature of biopsy	Site of biopsy	Percentage of lymphocytes positive	Degree of positivity
Rheumatoid arthritis:				
1	Synovium	R Knee	50%–90%	+ + +
2	Synovium	L Knee	50%–90%	+ + +
3	Synovium	R Wrist	50%–90%	+ +
4	Synovium	L Wrist	0%	−
5	Synovium	R Elbow	>90%	+ + +
6	Synovium	R Wrist	<50%	+
7	Synovium	R Knee	50%–90%	+ + +
8	Synovium	R Hand	0%	−
9	Synovium	L Knee	50%–90%	+ + +
Osteoarthritis:				
10	Synovium	R Hip	>90%	+ + +
11	Synovium	R Hip	>90%	+ + +
12	Synovium	L Hip	0%	−
13	Synovium	L Hip	50%–90%	+ +
14	Synovium	R Knee	50%–90%	+ +
15	Synovium	R Hip	<50%	+
Non-specific joint inflammation:				
16	Synovium	R Ankle	<50%	+
17	Synovium	R Knee	<50%	+ + +
18	Synovium	R Ankle	>90%	+ + +
Non-articular lesions:				
19	Skin		50%–90%	+ + +
20	Gallbladder		50%–90%	+ + +
21	Branchial Cyst		>90%	+ + +
22	Stomach (Gastritis)		>90%	+ + +
23	Ileum (Crohn's)		50%–90%	+ +

− = negative; + = weakly positive; + + = moderately positive; + + + = strongly positive.

the joint. The synovial fluid from patients with rheumatoid arthritis has been shown to contain interleukin-1,[23-25] granulocyte-macrophage colony stimulating factor,[26] macrophage colony stimulating factor[27] and a protein with similar properties to murine B cell growth factor 2.[28] In contrast, γ interferon[29] and interleukin-2[78] are found only in small quantities in the synovial fluid from these patients. Similar changes in the concentrations of these cytokines, however, are found in other chronic arthritides such as psoriatic and juvenile arthritis.[26] Thus changes in the concentrations of these cytokines are not specific to rheumatoid arthritis.

CD23 is a B lymphocyte activation marker and its soluble derivative has been shown to have effects on B cell growth and differentiation.[12] B cell activation has been suggested as an important pathogenic factor in rheumatoid arthritis, so CD23 is a candidate for involvement in the inflammatory cell infiltrate in the disease.

Many monoclonal antibodies have been raised against CD23, but are limited in their use as reagents for immunohistochemistry because they do not work on sections of paraffin wax embedded tissues. The antibody used in this study is unique in that it shows CD23 antigen in this type of specimen. Two distinct forms of the CD23 molecule have been shown, but these differ only in their intracytoplasmic regions.[30] BU38 recognises an epitope on the extracellular IgE binding part of the molecule, and so would be expected to bind to both forms of the membrane bound protein and to soluble CD23.

This study found CD23 positivity on lymphocytes in synovial biopsy specimens from most but not all patients with rheumatoid arthritis. There was no apparent correlation between the degree of CD23 staining and the activity of the disease as assessed by the ESR and the haemoglobin concentration. There was also no difference in CD23 staining between rheumatoid arthritis and other conditions showing chronic inflammation, both articular and non-articular. These findings suggest that CD23 expression is not of any specific pathogenetic importance in rheumatoid arthritis, but may be a characteristic feature of any chronic inflammatory response.

The finding of cytoplasmic endothelial staining in regions of CD23 positive cellular infiltrate is similar to that found in a previous study.[23] It is believed that this phenomenon is due to uptake of soluble CD23 by endothelial cells.

The results of this study suggest that CD23 is found in all forms of chronic inflammation

Sections showing chronic inflammatory cell infiltrate in synovial tissue stained with an immunoalkaline phosphatase method using the anti-CD23 primary antibody BU38. (A) A case of rheumatoid arthritis. Note that most lymphocytes show positivity for CD23. (B) A different case of rheumatoid arthritis that is negative for CD23.
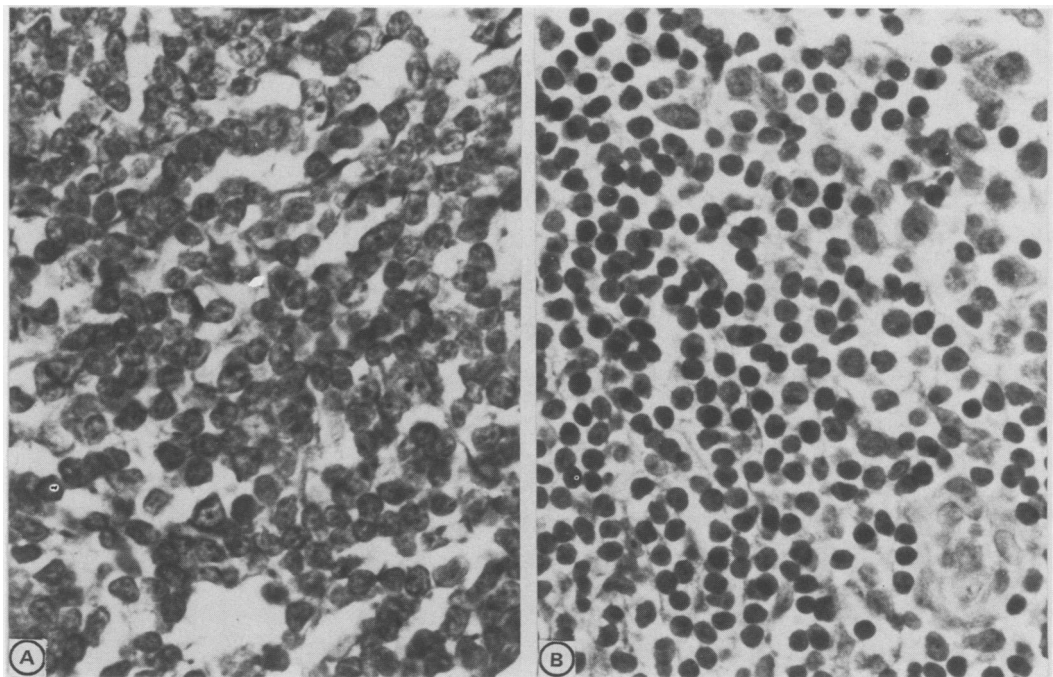
and is not likely to be specific for any particular form of chronic inflammatory disease. As CD23 was found on the surface of lymphocytes in many cases of these arthritides, however, sCD23 in serum or synovial fluid may still be a useful marker for the severity of the inflammatory infiltrate.

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