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Anti-keratin antibodies in patients with juvenile idiopathic arthritis

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ABSTRACT

Objective

We discuss the presence of anti-keratin antibodies (AKA) of the IgG class in patients with defined juvenile idiopathic arthritis (JIA).

Methods

An indirect immunofluorescence test with rat oesophagus substrate was used for the detection and quantification of AKA antibodies in patients' sera.

Results

Overall 30/60 patients with JIA had sera positive for AKA (50%, p=0,0005) ranging from 1:20 to 1:160 dilutions. Using the classification criteria for childhood idiopathic arthritis, AKA occurred in 2/7 patients with systemic disease (28.6%), in 13/30 patients with RF negative polyarthritis (43.3%, p=0.008) and in 12/18 RF positive polyarthritis (66.7%, p=0.002). AKA were also found in a small cohort of patients with oligoarthritis (1/3) and psoriatic arthritis (2/2). AKA positivity occurred in 3/26 healthy controls at a 1:20 dilution. The presence of AKA was correlated as well as with the severity of the disease. Our study revealed that AKA was present overall in 16/29 patients (55.2%) with severe JIA and in 11/26 patients (42.3%) with non-severe disease. We also observed that AKA remained positive regardless of disease activity. AKA were detectable in 44.4% patients with active JIA and in 45.9% patients in the complete or near remission.

Conclusion

Our data suggest that AKA are present in patients with JIA. However, no correlation with severity or disease activity was observed.

Introduction

Rheumatoid arthritis is the most prevalent systemic rheumatic disease, affecting 1-2% of the population. It is characterised by mononuclear cell infiltration and proliferation in the synovial membrane causing an irreversible degradation of the joint cartilage and bone structure (1). The etiology of rheumatoid arthritis remains unresolved, although various circulating autoantibodies directed to self-antigens have been found in patient sera. Rheumatoid factor (RF) is present in 70-90% of patients with RA and is included in the ARA classification criteria (2). Anti-keratin antibodies staining the stratum corneum of rat oesophagus, as described in RA patients in 1979 by Young *et al*. (3), have been shown to be the most specific serological marker for the diagnosis of RA, although it is less sensitive than rheumatoid factor. Anti-perinuclear factor (APF), as described by Nienhuis and Mandema in 1964 (4), reacting with an antigen in the keratohyaline granules of human buccal mucosa cells was later demonstrated to have the same specificity as AKA (5). Both AKA and APF reacts with the epitopes of fillaggrin, a filamentous protein located in the human epidermis, and with other fillaggrin-related proteins of various epithelial tissues (6,7). The goal of the present study was to test the presence of AKA in a cohort of patients with established juvenile idiopathic arthritis (JIA).

Patients and methods

60 patients (28 male and 32 female) aged 4-44 years (median 18.5 years) from the Outpatient Department of Rheumatology, University Hospital Motol in Prague were analysed for the presence of anti-keratin antibodies in serum samples. The underlying diseases, using the Idiopathic Arthritides of Childhood Classification criteria (8), were systemic arthritis (n=7), RF-negative polyarthritis (n=30), RF-positive polyarthritis (n=18), oligoarthritis (n=3) and psoriatic arthritis (n=2). Patients met the standard ACR (American College of Rheumatology) criteria for disease activity measures (9) and were divided into two groups depending on disease activity: 1) complete or near remission with or without on-going treatment, and 2) active disease. Patients were treated depending on the stage of the disease with non-steroid antirheumatics (NSAIDs), corticosteroids (C) and disease-modifying anti-rheumatic drugs (DMARDs). Twenty-six healthy controls (15 male and 11 female) aged 21-50 years (median 25.5 years) were included in the study.

An indirect immunofluorescence anti-

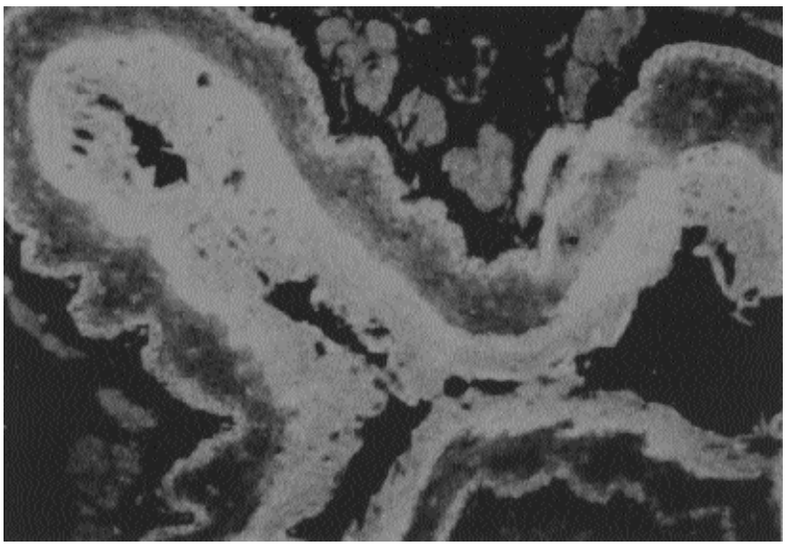

Fig. 1. Anti-keratin antibody of the IgG class on rat oesophagus substrate (magnification 200x).

body test (IIF) for the detection and quantification of anti-keratin antibodies in human serum was used (Immu-Glo™, Immco Diagnostics, Buffalo, USA). The IIF test was performed to the manufacturer's instructions. In brief, patients'sera at dilutions ranging from 1:10 to 1:320 were incubated on rat oesophagus sections for 30 minutes at room temperature. Any antibodies not bound to the substrate were removed by rinsing with phosphate buffered saline (PBS). Bound antibodies of the IgG class were detected by incubation (30 minutes at room temperature) with fluorescein-labeled, goat anti-human polyvalent IgG (FITC-conjugate). Reactions were observed under a fluorescence microscope (BX-50, Olympus, Japan) equipped with a wide band blue fluorescence cube for FITC (U-MWB, 450-480 nm) at a magnification of 200x or greater. Positive as well as negative controls were included with each test run. The results of the test for AKA were reported as positive with a titer ≥ 1:20 (Fig. 1).

The Fisher exact test was used for the statistical analysis.

Table I. The frequency of anti-keratin antibodies in patients with juvenile idiopathic arthritis and healthy volunteers.

AKA (titer)	Overall	Number of patients with JIA: Systemic disease	Polyarthritis RF negative	Polyarthritis RF positive	Oligo-arthritis	Psoriatic Arthritis	Healthy donors
Negative	30	5	17	6	2	0	23
1:20	12	1	4	6	0	1	3
1:40	13	1	6	5	1	0	0
1:80	4	0	2	1	0	1	0
1:160	1	0	1	0	0	0	0
≤ 1:320	0	0	0	0	0	0	0
	60	7	30	18	3	2	26
% AKA +	50	28.6	43.3	66.7	-	-	11.5
Fisher test	p = 0.0005	-	p = 0.008	p = 0.0002	-	-	

Results

Overall 30 out of 60 patients with JIA had sera positive for anti-keratin antibodies (AKA) of the IgG class (50%, p = 0.0005) ranging from 1:20 to 1:160 dilutions. Following the childhood classification criteria for idiopathic arthritis, anti-keratin antibodies occurred in 2 out of 7 patients with systemic disease (28.6%), in 13 out of 30 patients with RF negative polyarthritis (43.3%, p = 0.008), and in 12 out of 18 RF positive polyarthritis (66.7%, p = 0.002). AKA were also found in a small cohort of patients with oligoarthritis (1/3) and psoriatic arthritis (2/2). AKA positivity occurred in 3 out of 26 healthy controls at a 1:20 dilution. Another 23 healthy volunteers had serum negative for AKA antibody (Table I).

When we correlated the presence of AKA antibodies with disease activity, we found that antibodies were present in 8 out of 18 patients with active JIA (44.4%) and in 17 out of 37 patients in the complete or near remission group (45.9%). The lack of a significant difference between these two groups of patients confirmed that AKA remains positive regardless of disease activity (Table II).

Our study revealed that AKA was present overall in 16 out of 29 patients (55.2%) with severe JIA and in 11 out

Table II. Anti-keratin antibodies and disease activity in patients with juvenile idiopathic arthritis.

AKA (titer)	Overall		Systemic disease		RF negative polyarthritis		RF positive polyarthritis	
	Remission	Active disease	Remission	Active disease	Remission	Active disease	Remission	Active disease
Negative	20	10	4	1	13	6	3	3
Positive	17	8	2	0	6	5	9	3
	37	18	6	1	19	11	12	6
Fisher test	p = 0.65		p = 1		p = 0.75		p = 0.94	

Table III. Anti-keratin antibodies and severity of the disease in a cohort of patients with juvenile idiopathic arthritis.

AKA (titer)	Overall		Systemic disease		RF negative polyarthritis		RF positive polyarthritis	
	Severe	Non-severe	Severe	Non-severe	Severe	Non-severe	Severe	Non-severe
Negative	13	15	4	1	7	10	2	4
Positive	16	11	1	1	8	5	7	5
	29	26	5	2	15	15	9	9
Fisher test	p = 0.24		-		p = 0.23		p = 0.30	

of 26 patients (42.3%) with non-severe disease; however, this did not reach statistical significance (p=0.24) (Table III). Nevertheless, a higher titer of AKA positivity was observed in patients with severe disease.

Discussion

Anti-keratin antibodies, initially described by Young *et al*., were found to be highly specific for RA (3). The proportion of patients with RA who have AKA varies in different studies (10). Mallya *et al*. reported AKA positivity in 69% of patients with classical or definite RA (11), and Sharma *et al.* in 56% of studied RA cases (10). Significantly lower frequencies of AKA (32.9 - 38%) have been found in cohorts of patients with early RA compared with established RA patients (12, 13).

Our data showed a higher overall incidence of AKA in 30 out of 60 studied patients with established JIA (50%, p = 0.0005) when compared with the study by Gabay *et al*. on 124 children with different onset patterns of juvenile chronic arthritis (JCA), where antikeratin antibodies were detected only in 27% of all studied patients (16).

Using the indirect immunofluorescence on oesophagus sections, we observed AKA positivity in 66.7% of patients with RF positive polyarthritis (12/18, p= 0.002), in 43.3% of patients with RF negative polyarthritis (13/30, p = 0.008) and in 28.6% of patients with systemic disease. However, the differences between the categories did not reach statistical significance. Like Gabay *et al*. (16), we confirmed that AKA could not be considered specific for any subset of JCA as the percentages of serum samples positive for AKA did not differ significantly across JIA subgroups.

On the contrary, unlike Gabay *et al*. (16) who observed a significantly greater proportion of AKA only in RF negative polyarthritis patients (10 of 42 cases) compared to healthy controls, we found that AKA positivity was significantly higher in patients with seronegative as well as seropositive polyarthritis. However, the small cohort of 7 seropositive polyarthritis patients studied may not to significantly influence the final result. Meyer *et al*. also detected AKA by the same IIF on rat oesophagus sections in 58% of seropositive and 41% of seronegative rheumatoid polyarthritis patients (14).

In our cohort of patients with JIA, the presence of AKA was not correlated with the severity of the disease. This data confirms similar findings by Gabay *et al*. (16) in patients with JCA and by Sharma *et al.* (10), Mallya *et al.* (11) and Meyer *et al*. (14) in cohorts of patients with RA. Our study revealed that AKA frequency was lower in the group of patients with non-severe disease than in patients with severe JIA; however, this difference did not reach statistical significance (p = 0.24).

Cordonnier *et al*. found that AKA and/or APF did not become negative in RA patients on second-line drug therapy with methotrexate, gold, sulphasalazine, hydroxychloroqiune or corticosteroids, despite the loss of RF positivity and the achievement of well-controlled joint disease (15). Similarly, we observed that AKA remained positive regardless of disease activity. AKA were detectable in 44.4% patients with active JIA and in 45.9% patients in the complete or near remission. These data confirmed as well as the results of Gabay *et al*. study (16) done on 124 JCA patients concerning no relation of AKA status to disease activity.

We conclude that the presence of AKA is not specific to any JIA patient category. Futhermore, no relation between the presence of AKA and disease severity or activity was found.

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