

Basophil Histamine Release by RNA, DNA and Aggregated IgG Examined in Rheumatoid Arthritis and Systemic Lupus Erythematosus

Results Compared with Basophil Counts and Antinuclear Antibodies

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Basophils from patients with rheumatoid arthritis (RA) respond to RNA, DNA and immune complexes (aggregated IgG) with histamine release. The RNA response was well correlated to the clinical activity of the disease, since histamine liberation was found in all patients with severe activity, whereas no liberation was observed in patients with moderate or quiescent activity. A less significant correlation was obtained with DNA and aggregated IgG. In contrast, no response was obtained with RNA, DNA and aggregated IgG in patients with systemic lupus erythematosus (SLE) or in controls. In the RA and the SLE groups no significant correlation was found between the response of RNA, DNA and aggregated IgG and the serum titres of anti-DNA and antinuclear antibodies. No difference in basophil cell count in peripheral blood and basophil histamine content was found between RA, SLE and controls. Our results point to an involvement of an autoimmune type I reaction in the pathogenesis of RA directed against the nuclear components RNA and DNA and against immune complexes.

Key words: allergic type I reaction; antinuclear antibodies; basophil leucocytes; DNA; histamine release; rheumatoid arthritis; RNA; systemic lupus erythematosus.

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Immunological processes involving the allergic type I reaction may be important in the pathogenesis of rheumatoid arthritis (RA). Thus, elevated values of the total concentration of IgE in serum and of blood eosinophil counts are found in this disease (7, 11, 20).

In the present study we have investigated a possible involvement of an autoimmune type I reaction in RA directed against the nuclear components RNA and DNA and against immune complexes, i.e. aggregated IgG. The involvement was elucidated by the existence of basophil histamine release on exposure to

these "autoantigens". The investigation also included patients with systemic lupus erythematosus (SLE).

PATIENTS

Rheumatoid arthritis

Blood samples were collected from 19 patients with classical rheumatoid arthritis assessed by the criteria of the American Rheumatism Association (14). None of the patients revealed signs of atopic diseases. They comprised 15 women and four men,

ranging from 28–81 years of age (mean 66 years). The duration of the disease varied from 3 to 33 years (mean 15 years). Disease activity was graded on the basis of the clinical signs of joint inflammation and swelling. They were divided into three groups: quiescent, moderate, and severe activity, i.e. without joint swelling, with affection of one, and of two or more joints, respectively. Three patients were treated with sodium aurothiosulphate, four with prednisone, and two with azathioprine and one with penicillamin and prednisone. Fourteen patients had been treated with gold for at least 1 year earlier.

Systemic lupus erythematosus

Blood samples were obtained from 10 patients with SLE (3) in the active phase, all with at least one of the following manifestations: renal impairment, serositis, anaemia (Hb <6 nmol/ml), leukopenia (white cell count <2 × 10⁶/ml), thrombocytopenia (platelet count <50 × 10⁶/ml), or neurological symptoms. None of the patients revealed signs of atopic diseases.

The patients comprised nine women and one man ranging from 13–79 years of age (mean 52 years). The duration of the disease was 1–14 years (mean 5 years). Four patients were treated with prednisone, one with azathioprine and two with both azathioprine and prednisone, and one with levamisole.

Controls

Fourteen in-patients (12 women and 2 men), hospitalized for non-autoimmune and non-atopic diseases, ranging from 30–91 years of age (mean 64 years) and eight normal persons (5 women and 3 men) ranging from 28–42 years of age (mean 34 years) were used as controls.

METHODS

Cell suspension

8.5 ml blood samples were collected by venipuncture in plastic tubes containing

0.5 ml 0.2 M EDTA (ethylene diamine tetraacetic acid) (pH 7.2). 22.5 ml 0.9% NaCl was added to each sample. The leukocytes were isolated according to the method of Day (4). 10 ml Ficoll-Hypaque solution (12.8 g Ficoll + 21 g Hypaque, i.e. sodium diatrizoate, H₂O is added up to 190–200 ml, the final solution having a density of 1.080 g/ml at 20°C) was placed below the mixture. The sample was centrifuged at 400 g for 40 min and the cotton wool-like interphase consisting of monocytes with approx. 2% basophils was transferred to a plastic tube containing 15 ml Tris-AMC (tris (hydroxymethyl) amino-methane 25 mM at pH 7.6, NaCl 0.12 M, KCl 5 mM, MgCl₂ 1.15 mM, CaCl₂ 0.6 mM, human serum albumin 0.3 mg/ml, and glucose 1 mg/ml). The mixture was centrifuged at 107 g for 10 min and the cell sediment was resuspended in 10 ml Tris-AMC. The suspension was centrifuged at 60 g for 10 min and the cell sediment was resuspended in 7.5 ml Tris-AMC. The cell suspensions were then pooled.

Basophil histamine release

400 µl cell suspensions containing 2,000–8,000 basophils were incubated in plastic tubes at 37°C for 40 min with 20 µl antigen at different concentrations, or with sodium aurothiosulphate. Histamine release was determined fluorometrically according to the method of Shore et al. (15), omitting the extraction procedure according to Bergendorff & Uvnäs (2). 3 ml ice-cold Tris-AMC was added to the samples, which were then centrifuged at 2,000 g for 10 min at 4°C. The supernatant contained released histamine and protein. Since the latter interferes with the histamine analysis the supernatant was discarded and the residual histamine was determined in the cell sediment according to Stahl Skov & Norn (17).

The release of histamine was expressed as a percentage of the total histamine content of the sample. Correction was made for the spontaneous histamine release amounting to up to 10%.

Antigens

DNA (No. D-1501, Sigma, U.S.A.), RNA (No. R-7250, Sigma, U.S.A.) and aggregated IgG were used in final concentrations of 0.05 μ g, 5 μ g, 0.5 ng, 50 ng, and 5 μ g per ml. DNA was dissolved in Tris-AMC by exposure to 37°C for 3 h. Aggregated IgG was prepared by heating 10 mg human IgG (Kabi, Sweden) in 10 ml Tris-AMC for 40 min at 61°C.

In a few experiments RNase digestion of RNA was carried out to exclude the possibility of histamine release caused by impurities. RNA in final concentrations of 5 μ g/ml was incubated for 24 h at 37°C with 100 μ g/ml RNase (R-4875, Sigma, U.S.A.) in Tris-AMC with 10 mM $MgCl_2$ and then examined for histamine-releasing activity.

Sodium aurothiosulphate

The possibility of histamine release caused by gold therapy was elucidated by exposure of the cell suspension to sodium aurothiosulphate (Ferrosan A/S, Denmark) in final concentrations of 10 ng and 10 μ g per ml.

Basophil count and histamine content

Basophils were stained and counted according to the method of Kimura et al. (8) with the minor modification that the blood and cell suspensions were diluted 1:5 and 1:2, respectively, with the staining solution. The content of histamine per basophil was determined in the cell suspension instead of in blood, since the histamine analysis is only specific for determining histamine in the cell suspension.

Sera

Sera were investigated for the occurrence of granulocyte-specific and organ-non-specific antinuclear antibodies (GS-ANA and ON-ANA) and of anti-DNA antibodies, all belonging to IgG and IgE classes. Indirect immunofluorescence technique was used.

Smears of isolated and washed human leukocytes as well as rat liver cryostat sections served as nuclear substrates for antinuclear antibodies (18, 19), and the kinetoplasts of *Crithidia luciliae* served as DNA substrate (12). Furthermore, complement C3-fixing properties of these antibodies (CF-ANA) were investigated (18, 19). Sera were screened 1:16 for IgG and undiluted for IgE and complement-fixing properties.

The following conjugates were used: Fluorescein isothiocyanate (FITC)-labelled rabbit IgG specific for human γ chain and β 1c component of human C3 were from Dakopatts (Denmark). Rabbit antisera specific for human ϵ chain were obtained from Behringwerke (GFR). The isolated IgG fraction was labelled with FITC (19). The antisera showed monospecific reactions in crossed immunoelectrophoresis. FITC-labelled IgG and IgE were tested for specificity by means of IgA, IgM, IgG, IgD and IgE monoclonal bone marrow specimens from patients with multiple myeloma and macroglobulinaemia (19). The fluorescein/protein ratios estimated by optical density 495/280 nm were 0.5–0.7. The sections were examined in a Leitz Orthoplan fluorescence microscope equipped for incident light illumination using an Osram 200 mercury lamp as light source (18).

Rheumatoid factors were demonstrated by the FII Latex (Behringwerke, GFR) fixation slide test and the Waaler-Rose sheep cell agglutination test (W-R). Titres ≥ 32 and ≥ 40 , respectively, were considered abnormal. LE cell test was studied by the method of Hammer (6).

The total concentration of IgE in serum was determined by the paper-radioimmunosorbent technique (PRIST) using Phadebas IgE kits from Pharmacia (Sweden). The concentrations were expressed in U according to WHO standard serum (1 U = 2.4 ng IgE). Serum concentrations of IgG, IgM, IgA and IgD and α_2 -macroglobulin were determined by quantitative immunoelectrophoresis (1) using

Table 1
Parameters in patients with rheumatoid arthritis

RA group	Patient No.	RNA	Histamine release ^a DNA	H/B	B/ μ l	E%	ESR	GS-ANA ^b	ON-ANA ^b	CF-ANA	Anti-DNA ^b	W-R	Latex test	Total IgE
Severe activity	1	+	+	0.45	64	1	58	16						20
	2	+	+	2.20	25	2	44	256	32	32				43
	3	+	+	0.55	41	9	112	16	(8)		16	+	+	2
	4	+	+	1.49	11	10	89		512	32	32	+	+	15
	5	+	+	1.83	49	2	72		(16)		(4)			
	6	+	+	0.72	39	1	70		16			+	+	130
	7	+	+	0.95	27	0	125		128			+	+	1
	8	+	+	?	?	0	88	16				+	+	10
Moderate activity	9	-	+	1.07	14	5	6	256	64	8		+	+	15
	10	-	+	0.55	20	0	15		16	4	(16)		+	70
	11	-	-	0.74	36	1	67	64	16			+	+	170
	12	-	-	0.58	55	6	38	128		4	64			95
Quiescent activity	13	-	-	1.12	55	0	72		32	8			+	20
	14	-	-	1.10	22	4	3		(4)					
	15	-	-	0.45	63	3	68		64	4				485
	16	-	-	0.24	75	0	30		32			+	+	80
	17	-	-	0.78	52	5	45					+	+	155
	18	-	-	0.96	49	3	38	64				+	+	270
	19	-	-	0.79	49	3	4	256	64	8		+	+	44
												+	+	15

H/B: histamine content (pg base) per basophil; B/ μ l and E%: cell counts in peripheral blood of basophils and eosinophils; GS-ANA, ON-ANA and CF-ANA: granulocyte-specific and organ-non-specific antinuclear antibodies and complement C3-fixing properties of these antibodies; W-R: Waaler-Rose sheep cell agglutination test.

^a + histamine release; - no release.

^b Serum titres of IgE (<1) in brackets and of IgG ($\geq$ 16) without brackets.

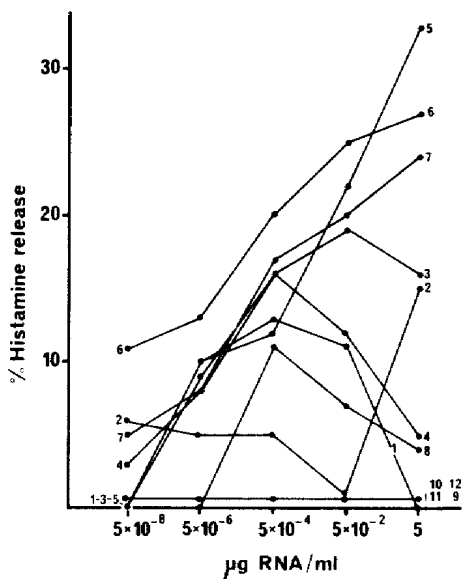


Fig. 1. Basophil histamine release caused by RNA in rheumatoid arthritis of severe and moderate activity. Patients' numbers are stated on the curves.

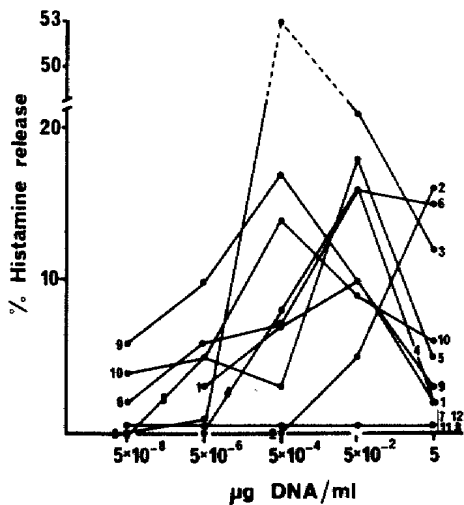


Fig. 2. Basophil histamine release caused by DNA in rheumatoid arthritis of severe and moderate activity.

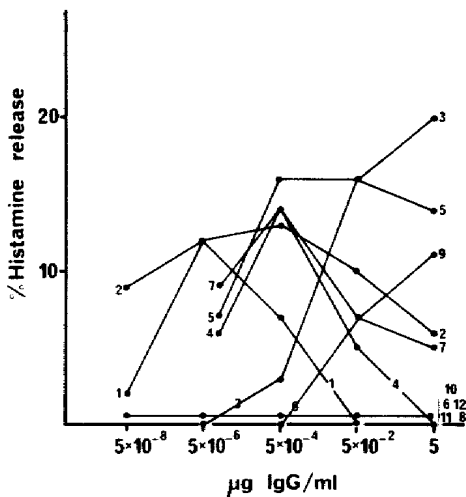


Fig. 3. Basophil histamine release caused by aggregated IgG in rheumatoid arthritis of severe and moderate activity.

monospecific conjugate from Dakopatts and Behringwerke. Normal values amount to: IgE 1–110 U/ml; IgG 7.2–15.1 mg/ml; IgM 0.23–1.33 mg/ml; IgA 0.74–3.06 mg/ml; IgD < 50 U/l, and α_2 -macroglobulin ♀ 1.83–4.62, ♂ 1.32–3.90 mg/ml.

RESULTS

Table 1 shows 19 patients with RA divided into three groups: quiescent, moderate, and severe activity. All patients with severe activity responded to RNA with basophil histamine release, whereas no response was observed in patients with moderate and quiescent activity. DNA-response was found in six of eight patients with severe activity and in two of four with moderate activity, whereas no histamine release was observed in the quiescent group. Similar results were obtained by aggregated IgG with the exception that in the group with moderate activity, only one patient showed histamine release.

The dose-response curves for histamine release by RNA, DNA and aggregated IgG are shown in Figs. 1–3. In the

semilogarithmic plot, increasing concentrations of these antigens caused an increased histamine liberation, which was maximal above 0.5 ng per ml cell suspension. No correlation was found in maximal release of histamine by RNA, DNA and aggregated IgG. RNase digestion of RNA was carried out in patients 4 and 7 (Fig. 1) before the cells were exposed to 5 µg/ml RNA. Hereby the histamine release was completely abolished.

Table 2 shows 10 patients with SLE in the active phase. None of these patients responded to RNA, DNA or aggregated IgG. The same applied to the controls (not shown).

No histamine release was obtained by sodium aurothiosulphate in RA patients, SLE patients or in the controls.

The patient population was extended for determination of basophil counts and histamine content. Basophil count in peripheral blood was found to be 40 ± 22 (s.d.) per µl in 21 normal individuals. In SLE patients in the active phase and in RA patients with severe, moderate, and quiescent activity the mean values were 33 ± 14 ($n=10$); 37 ± 17 ($n=10$); 31 ± 18 ($n=4$) and 47 ± 15 ($n=11$), respectively. These values do not differ from one another or from those of the control group ($P>5\%$ by Student's *t*-test). The histamine content expressed as pg histamine base per basophil was calculated to 0.80 ± 0.31 ($n=12$) in controls, 0.97 ± 0.50 ($n=10$) in SLE patients in the active phase, and 1.17 ± 0.68 ($n=7$); 0.87 ± 0.27 ($n=4$) and 0.70 ± 0.30 ($n=7$) in RA patients with severe, moderate and quiescent activity, respectively. These values do not differ from one another.

In the RA group (Table 1) and SLE group (Table 2) no significant correlation was found in the single patient between the response of RNA, DNA and aggregated IgG and the other parameters including the serum titres of antinuclear antibodies and anti-DNA, total serum IgE and the immunoglobulins IgG, IgM, IgA, and IgD and α_2 -macroglobulin (not shown) and Waaler-Rose

and Latex test, erythrocyte sedimentation rate and cell counts in peripheral blood of eosinophils and basophils.

The RA group revealed a tendency to higher total serum IgE and eosinophil counts than the SLE group and the controls. In the control group total IgE amounted to 45 U/ml ranging from 1–110 and eosinophils amounted to 1% ranging from 0–5%.

A tendency to higher erythrocyte sedimentation rate (ESR) was found in RA patients with severe activity and in SLE patients in the active phase. The group of RA patients showed a higher tendency to GS-ANA of IgG class, Waaler-Rose and Latex test, and a higher tendency was found in the SLE group to ON-ANA of IgG class, complement-fixing properties and anti-DNA. Nineteen patients with RA revealed hyperimmunoglobulinaemia of IgG in five, of IgM in three, and of IgA in none, and one had a low level of IgA. The 10 SLE patients revealed high serum IgG in seven, of IgA in one and one had a low level of IgA. All patients had normal values of serum IgD and α_2 -macroglobulin. Positive LE cell test was only obtained in the SLE group, where four patients responded with positive reaction.

DISCUSSION

It is well-known that type III reactions play a role in RA and SLE (13). However, the allergic type I reaction might also be involved in the pathogenesis of RA. Thus, elevated values of serum IgE and blood eosinophil counts have been found in this disease (7, 11, 20).

In the present study the possible involvement of an autoimmune type I reaction in RA directed against the nuclear components RNA and DNA, and against immune complexes, was investigated. In the type I reaction antigen reacts with antibody bound to mast cells and basophils, and this interaction leads to release of histamine. Histamine release thus indicates a type I reaction. Basophil histamine release technique was used, as this method is sensitive for detecting

Table 2
Parameters in patients with systemic lupus erythematosus in active phase

Patient No.	RNA	Histamine release ^a DNA	H/B	B/ μ l	E%	ESR	GS-ANA ^b	ON-ANA ^b	CF-ANA	Anti-DNA ^b	W-R	Latex test	Total IgE
20	-	-	0.34	33	1	55		128 (4)	8				20
21	-	-	0.67	38	1	125		16	4				10
22	-	-	1.06	41	0	65		512 (16)	16	16			12
23	-	-	2.13	14	1	37		8192 (128)	512	128 (16)			10
24	-	-	0.89	16	1	57		2048	2048	16		+	51
25	-	-	1.05	31	0	21	128	32	32				17
26	-	-	0.63	23	0	125		256	32			+	12
27	-	-	0.66	33	0	20		16					16
28	-	-	1.40	61	0	21		128					1
29	-	-	0.86	44	0	65		256	64	32	+	+	56

For abbreviations see Table 1.

type I allergy (16). We found that basophils from RA patients responded to RNA, DNA and aggregated IgG with histamine release. The dose-response curves (Figs. 1–3) were similar to those obtained by the allergic type I reaction in grass pollen hay fever patients (16). The RNA response was well correlated to the clinical activity of the disease, since histamine liberation was found in all patients with severe activity, whereas no liberation was observed in patients with moderate or quiescent activity. A less significant correlation was observed with DNA and aggregated IgG. The RNA response was caused by the nucleotide and not by impurities, since a complete abolishment of histamine release was obtained by RNase digestion.

The study also included 10 patients with SLE in the active phase. These patients did not respond to RNA, DNA and aggregated IgG.

In the RA and SLE groups of patients no significant correlation was found between the response of RNA, DNA and aggregated IgG and the serum titres of antinuclear antibodies and anti-DNA in the single patient. Such a correlation was not necessarily to be expected, since basophil histamine release might be obtained by smaller amounts of antibodies than could be detected by indirect immunofluorescence technique, and since basophil cell reactivity might only exist in RA of severe or moderate activity.

In some patients with RA an increase in total serum IgE and in blood eosinophil counts was found, in contrast to the SLE group. These differences are in agreement with other authors (5, 7, 11, 20). Basophil cell counts in peripheral blood were found to be 40 ± 22 (s.d.) per μl in the control group, which is in accordance with Lorenz et al. (9) and Kimura et al. (8). Similar cell counts were found in RA and SLE patients.

The possibility of histamine release with gold therapy, caused by toxic or allergic reactions, was elucidated by exposure of the cells to sodium aurothiosulphate. No liberation of histamine was observed and this

result fits with the observation that gold does not release histamine, but, on the contrary, inhibits the anaphylactic histamine release, probably by inhibiting the antigen-antibody reaction (10).

Our findings of basophil histamine release in RA by the nuclear constituents RNA and DNA and by immune complexes point to an involvement of an autoimmune allergic type I reaction in the pathogenesis of RA. The type I reaction would explain the swelling phenomena of the joints with local inflammation, caused by release of histamine and other inflammatory mediators from mast cells and basophils. Morphological studies of tissue mast cells in the joint may reveal changes in morphology, cell counts, and histamine content in contrast to our findings of basophils in peripheral blood.

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