

Anti-interleukin-1 α autoantibodies in humans: Characterization, isotype distribution, and receptor-binding inhibition—Higher frequency in Schnitzler's syndrome (urticaria and macroglobulinemia)

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Since autoantibodies (Abs) to cytokines may modify their biologic activities, high-affinity binding factors for interleukin-1 α (IL-1 α BF) were characterized in human sera. IL-1 α BF was identified as IgG (1) by sucrose density-gradient centrifugation followed by immunodiffusion autoradiography, (2) by ligand-blotting method, (3) by ligand binding to affinity-immobilized serum IgG, and (4) by IgG affinity purification followed by sucrose density-gradient centrifugation. IL-1 α binding activity resided in the F(ab)₂ fragment. The apparent equilibrium constant was in the range of IgG found after immunization with conventional antigens (i.e., 10⁻⁹ to 10⁻¹⁰ mol/L). Anti-IL-1 α IgG auto-Abs represented only an extremely small fraction of total IgG (<1/10⁻⁵). Some sera with IL-1 α BF and purified IgG thereof were able to inhibit by 96% to 98% the binding of human recombinant IL-1 α to its receptor on murine thymoma EL4-6.1 cells, whereas other sera did not. When ¹²⁵I-labeled anti-IL-1 α IgG complexes were injected into rats, they prolonged the plasma half-life of ¹²⁵I-labeled IL-1 α several fold and altered its tissue distribution. The predominant class was IgG (12/19), mainly IgG4 (9/19), but in five of the sera, anti-IL-1 α IgA was also detected. In a screening of 271 sera, IL-1 α BF was detected in 17/98 normal subjects and was not more frequent in several control groups of patients, except in patients with Schnitzler's syndrome (fever, chronic urticaria, bone pain, and monoclonal IgM paraprotein) (6/9; p < 0.005). The pathologic significance of these auto-Abs remains to be determined. (J ALLERGY CLIN IMMUNOL 1991;88:244-56.)

Key words: IgA autoantibodies, IgG4 autoantibodies, interleukin-1 plasma clearance, Schnitzler's syndrome, urticaria, and macroglobulinemia

In a preliminary study of a patient affected by a rare syndrome characterized by fever, chronic urticaria, bone pain, and monoclonal IgM paraprotein (SS¹), we identified serum IgM with immunoreactivity

Abbreviations used

IL-1:	Interleukin-1
IL-1 α BF:	Interleukin-1 α binding factor
rh:	Recombinant human
SS:	Schnitzler's syndrome
TNF:	Tumor necrosis factor
Ab:	Antibody
PAGE:	Polyacrylamide gel electrophoresis
PBS:	Phosphate-buffered saline
K _m :	Apparent equilibrium constant (Michaelis-Menten constant)
PEG:	Polyethylene glycol

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against IL-1 α by immunoblotting analysis.² We thus attempted to characterize further auto-Abs to cytokines. This was also done because signs and symptoms of urticaria with systemic reactions, such as fever,

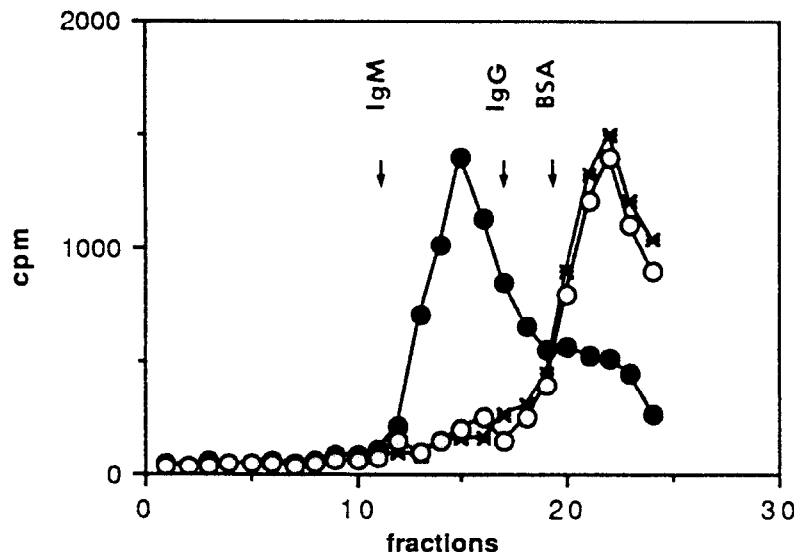


FIG. 1. IL-1 α BF of a patient with SS analyzed by sucrose density-gradient centrifugation. Serum was incubated with ^{125} I-labeled IL-1 α and centrifuged at 30,000 rpm for 16 hours; ^{125} I-labeled IL-1 α incubated with buffer alone (★); ^{125} I-labeled IL-1 α incubated with SS1 serum (●); and ^{125} I-labeled IL-1 α incubated with SS1 serum in presence of 2000-fold excess of cold IL-1 α (○). Arrows indicate the sedimentation of ^{125} I-labeled human IgM and IgG, and ^{125} I-labeled bovine serum albumin. Note: the incorporation of ^{125} I-labeled IL-1 α into large molecular weight complexes in the patient's serum, which was reversed by excess cold IL-1 α .

might involve IL-1 ^{3} and because Abs to cytokines might be able to attenuate or amplify the effect of these active biologic mediators by various mechanisms, such as altered tissue distribution. ^{4,5} The purpose of the present study was to identify the immunoglobulin classes and subclasses involved in IL-1 α binding activity, to analyze binding activity of F(ab) $_2$ fragments, to study interference of these immunoglobulins with binding of ^{125} I-labeled IL-1 α to its receptor, to investigate the effect of IL-1 α BF on plasma clearance and tissue distribution of IL-1 α in the rat, and to analyze IL-1 α BF in a large panel of sera from normal control subjects and patient groups with various inflammatory diseases chosen to mimic the signs and symptoms of SS.

PATIENTS AND METHODS

Methods

Reagents. Iodinated (^{125} I) rh IL-1 α and IL-1 β (specific activities, 2300 and 1600 Ci/mmol/L, respectively) were purchased from Amersham (Amersham International, Buckinghamshire, U.K.). ^{125} I-labeled rh TNF- α (specific activity, 1500 Ci/mmol/L) was a gift from Glaxo (Geneva, Switzerland).

For inhibition studies in the receptor-binding assay, rh IL-1 α (Glaxo) was iodinated according to the chloramine-T method, as previously described. ^{6} ^{125} I-labeled IL-1 α retained full biologic activity and had a specific activity of 3 to 5×10^5 cpm/ng.

Preparation of IgG and F(ab) $_2$ fragments. IgG and IgG-

depleted sera were obtained by passing 1 ml of serum three times over 5 ml of protein G-Sepharose columns (Pharmacia LKB, Uppsala, Sweden) previously equilibrated with 20 mmol/L of sodium phosphate buffer, pH 7 (starting buffer), eluted with 200 mmol/L of glycine HCL, pH 2.8 (elution buffer), and immediately neutralized with 1 mol/L of Tris buffer, pH 9.0. The IgG was concentrated to 10 mg/ml, and the IgG-depleted sera was concentrated to their original volume. F(ab) $_2$ fragments were obtained by digestion of 50 mg of IgG with 1 mg of pepsin (Sigma Chemical Co., St. Louis, Mo.) in sodium acetate buffer, pH 4.5, for 16 hours at 37 $^\circ$ C. The F(ab) $_2$ fragments were purified by gel filtration on Sephacryl S-200 (Pharmacia), and the undigested IgG contaminant was removed by chromatography on protein G-Sepharose. The purity of F(ab) $_2$ was tested by sodium dodecyl sulfate-PAGE under reducing and nonreducing conditions.

Binding studies. Binding studies were done by incubation of either 0.010 to 1 nmol/L of (5500 to 550,000 cpm) IL-1 α , 0.132 nmol/L of rh IL-1 β (48,000 cpm), or 0.132 nmol/L of TNF- α (45,000 cpm) for 1 hour at 37 $^\circ$ C in 100 μ l of PBS containing 0.03% PEG 6000 (Merck, Zurich, Switzerland) with serum at various dilutions, IgG, or F(ab) $_2$ fragments. The specificity of the IL-1 α binding was assessed by adding 2000-fold excess of unlabeled IL-1 α into the solutions. The binding reaction was analyzed with two procedures:

1. **Sucrose density-gradient centrifugation:** The samples were layered onto a continuous sucrose gradient (4.5 ml, 10% to 50% wt/vol in PBS) and run overnight at 30,000 rpm at 4 $^\circ$ C with a swing-out TST.55.5 Kontron

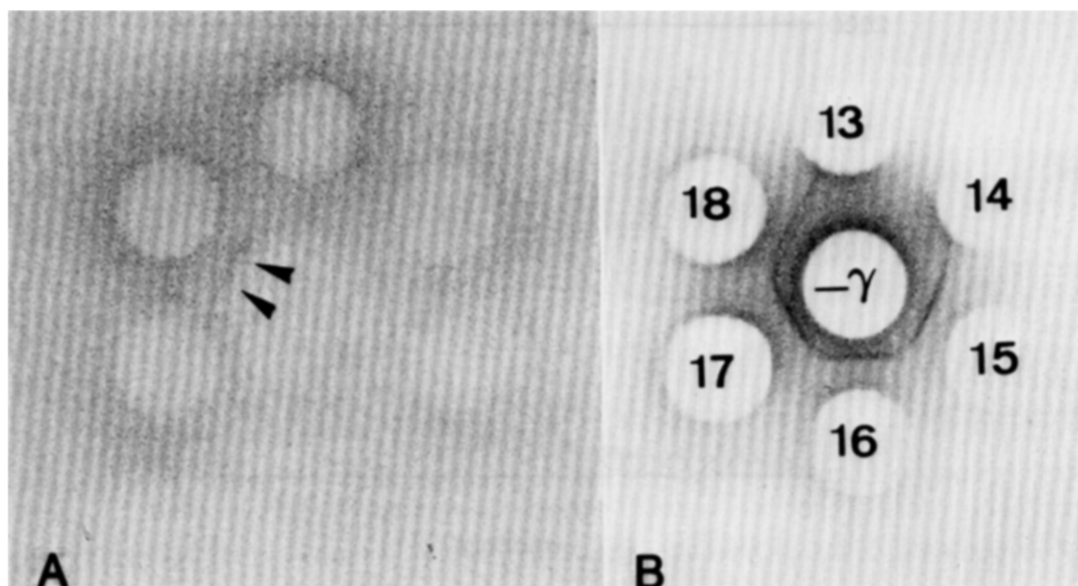


FIG. 2. Double immunodiffusion of sucrose density-gradient fractions (from 13 to 18; see Fig. 1) of SS1 serum revealed by antihuman IgG. **A**, Coomassie brilliant blue protein staining; IgG is localized in fractions 15 to 18. **B**, Autoradiography of **A**. A radioactive line in fraction 15 (arrows) indicating ^{125}I -labeled IL-1 α -IgG complex produced by the serum illustrated in Fig. 1.

rotor (Kontron, Basel, Switzerland). Fractions (200 μl) were collected ($n = 24$) and counted for radioactivity. Radiolabeled bovine serum albumin, IgG, and IgM served as 5.5 Svedberg's unit (S), 7S and 19S markers, respectively.⁷ By this technique, peaks separated by two fractions indicate a significant difference in size.⁷

2. **PEG precipitation:** Two hundred fifty microliters of 20% PEG in PBS was added to 100 μl sample, mixed, and centrifuged (2000 g) for 10 minutes at 4° C. The radioactivity of both the pellet and the supernatant was counted, expressed as percentage of precipitated radioactivity calculated after subtraction of percentage of nonspecific precipitation obtained with buffer alone ($8\% \pm 1\%$). The titer of IL-1 α BF, which represents the final dilution that precipitated $>10\%$ of ^{125}I -labeled IL-1 α was determined in sera diluted up to 1/320.

K_m was studied in 11 selected sera chosen for high IL-1 α BF activity (i.e., $>20\%$ PEG precipitation) to optimize the affinity studies: four from patients with SS, five from patients with other skin diseases, one from a kidney-graft patient, and one sera from a normal subject. One hundred microliters of serum was incubated for 1 hour at 37° C with 0.01, 0.02, 0.03, 0.05, 0.10, 0.50, and 1 nmol/L of ^{125}I -labeled IL-1 α , and was then precipitated with PEG, as described above. The apparent K_m was determined according to the Michaelis-Menten equation. All binding studies were done in duplicate or triplicate.

Ligand-blotting assay. Three microliters of sera was run in 5% PAGE in nondenaturing conditions and transferred onto nitrocellulose sheets. After blocking proteins in PBS containing 5% defatted milk (Régilait, France Lait, St. Martin-Belle-Roche, France), pH 7.2 (blocking buffer), the

nitrocellulose sheets were incubated overnight at 4° C with 0.132 nmol/L of ^{125}I -labeled IL-1 α in blocking buffer alone or with either 100-fold excess unlabeled IL-1 α or IL-1 β . After a wash in PBS (total, 1 L), the sheets were overlaid with x-ray films (X-OMAT, Kodak, Lausanne, Switzerland) and exposed at -70°C for 1 week. The positions of immunoglobulins were determined on separate nitrocellulose sheets revealed with peroxidase-labeled goat anti- μ or anti- γ human heavy immunoglobulin chains (Institut Pasteur, Paris, France).

Ligand binding to affinity-immobilized serum IgG. Sera were loaded onto protein G-Sepharose columns as described above. After an extensive wash with starting buffer, ^{125}I -labeled IL-1 α was added (50,000 cpm), washed, and finally eluted with elution buffer. Column effluent from the washing and elution steps was counted in a gamma counter.

Double immunodiffusion autoradiography. ^{125}I -labeled IL-1 α was incubated with undiluted serum or with the peak of IL-1 α BF from the sucrose gradient for 1 hour at room temperature to allow the formation of IL-1 α BF complexes. The samples were then added to wells in a 2% agarose gel (Ouchterlony's method) against various antisera (rabbit antihuman immunoglobulin γ , μ , and α heavy chains and κ and λ light chains from Behring [Hoechst-Pharma, Zurich, Switzerland]; sheep antihuman immunoglobulin $\gamma 1$ and $\gamma 2$ from Serotec [Inotech, Wohlen, Switzerland]; and anti $\gamma 3$ and $\gamma 4$ from Miles Scientific [Dr. R. Sreuli, Zurich, Switzerland]). The formation of IL-1 α BF complexes was checked by the colocalization of the precipitation line revealed by Coomassie brilliant blue staining of the gels and the lines produced on the x-ray film by autoradiography of the same gels (exposure time, 2 weeks at -70°C).

In vitro inhibition studies of IL-1 α receptor binding. Binding of ^{125} I-labeled IL-1 α was performed on the murine thymoma EL4-6.1 cells, as previously described.⁶ Either sera or IgG fractions were incubated with ^{125} I-labeled IL-1 α for 1 hour at 37° C before adding to the EL4-6.1 cells.

IL-1 α clearance studies in rats. Rats were injected intravenously with PBS containing ^{125} I-labeled IL-1 α (400,000 cpm) and 20 mg of IgG from different subjects. The solutions were incubated for 15 minutes at room temperature before intravenous injection into anesthetized animals (protocol approved by the local Ethical Committee for Animal Studies). Blood samples (500 μ l) were obtained at various time intervals (from 30 seconds to 60 minutes) and treated with heparin. Plasma and cells were separated by centrifugation at 10,000 g for 3 minutes. The protein-bound radioactivity was determined in the trichloroacetic acid precipitate (final concentration, 100 gm/L). An elimination curve was constructed with the 30-second sample as 100% value. The plasma volume calculated from these samples was in the expected range between 34.5 and 46 ml/kg of body weight. The animals were killed, and the radioactivity was measured 60 minutes after injection in different tissues and organs (skin, muscle, kidney, liver, spleen, and lung). The results are expressed as percentage of total injected radioactivity per gram of wet tissue. Each determination was done in triplicate.

Patients and control subjects

Sera from 271 subjects were analyzed. The sera of nine patients with SS were investigated, four female and five male patients, mean age, 63 years (range, 50 to 80 years). The clinical and biologic data from five of these patients have previously been published.^{1, 8-11}

Because it was difficult to define a single proper control group for patients with SS, we decided to test sera from patients with a wide variety of diseases, each of which was chosen to match the signs and symptoms occurring in patients with this syndrome. We included sera from (1) nine patients with inflammatory skin diseases and monoclonal immunoglobulin without criteria for lymphoproliferative disease, (2) two patients with monoclonal IgM without inflammatory skin symptoms and no other systemic diseases, and (3) 90 patients with various inflammatory dermatoses but without monoclonal immunoglobulin. This group included 72 patients with extensive chronic pruritic dermatoses (nonatopic eczema), 13 with bullous pemphigoid, two with chronic urticaria, and three patients with delayed-pressure urticaria. Ten patients who had received a kidney transplant and were currently receiving systemic steroid therapy were included because such therapy was administered to both the group with SS and the group with various inflammatory skin diseases. Thirty patients, admitted to the hospital for various diseases but without inflammatory skin disorder, older than 80 years (15 male and 15 female patients), were also studied to determine if the frequency of IL-1 α BF increases in elderly patients, 28 patients with diseases accompanied by chronic or intermittent fever (systemic lupus erythematosus, 15; cryoglobulinaemia, 7;

AIDS, 6) and 93 normal healthy control subjects, including 86 blood donors and seven subjects working in the hospital laboratories.

Statistics

The Mann-Whitney U test and the Fisher's exact test were performed when this was appropriate.

RESULTS

Detection and characterization of IL-1 α BF

The serum from a patient with SS (subsequently referred to as SS1) was incubated with ^{125} I-labeled IL-1 α , ^{125} I-labeled IL-1 β , or ^{125} I-labeled TNF- α to determine whether it contained factor(s) binding these cytokines. When IL-1 α was analyzed by the sucrose gradient assay, the position of the IL-1 α peak was displaced by the SS1 serum, indicating the formation of a complex between IL-1 α and the SS1 serum (Fig. 1). The complex had a sedimentation constant between 7S and 19S. All sera from this patient obtained during a 3-year period provided identical results (data not presented). Dilution of the serum reduced the peak size of complexed IL-1 α . No displacement of the ^{125} I-labeled IL-1 α peak was observed in the presence of excess cold IL-1 α , demonstrating the specificity of complex formation between a serum factor and IL-1 α (Fig. 1). ^{125} I-labeled IL-1 β and ^{125} I-labeled TNF- α were not displaced by the same serum data (not presented).

The fractions of the sucrose density-gradient assay containing the IL-1 α -IgG complex from the SS1 serum illustrated in Fig. 1 were studied by double immunodiffusion against antihuman IgG and antihuman IgM (data not presented). The precipitation line of IgG/anti-IgG colocalized with radiolabeled IL-1 α provided evidence that the IL-1 α BF of this serum corresponds to IgG (Fig. 2).

Ten sera from normal control subjects were then studied by the sucrose density-gradient assay. One sera (subsequently referred to as NHS1) was demonstrated to contain an IL-1 α BF; the remaining nine sera did not (data not presented). However, the sedimentation rate of the complex formed by NHS1 serum was larger than that formed by the SS1 serum, suggesting the presence of a different type of IL-1 α BF. Thus, both sera were depleted of IgG with protein G. IgG-depleted SS1 serum no longer demonstrated IL-1 α binding activity (Fig. 3), indicating that the BF was only IgG. By contrast, IgG-depleted NHS1 still bound a fraction of IL-1 α (Fig. 3), suggesting that this serum contained at least two different IL-1 α BF.

The binding of IL-1 α was then characterized by a ligand-blotting assay (Fig. 4). After electrophoresis

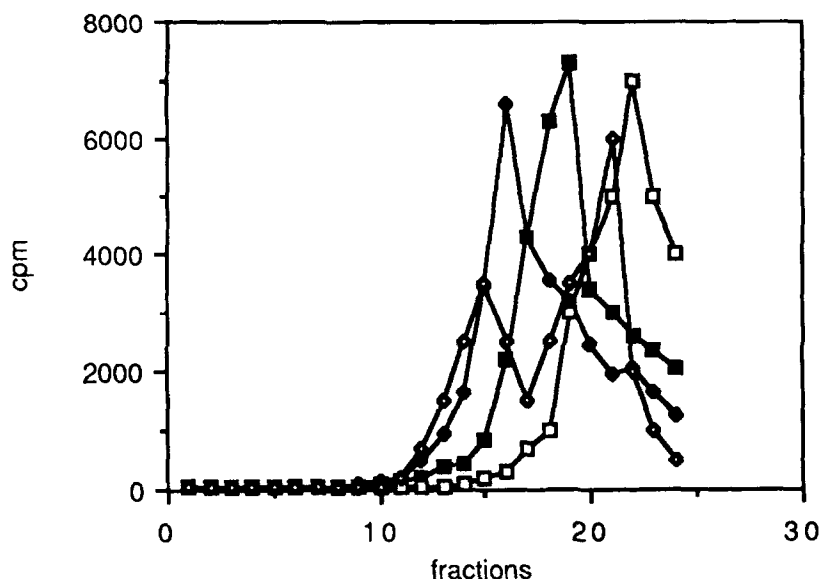


FIG. 3. Different IL-1 α BFs in a normal human serum (NHS1) illustrated by sucrose density-gradient centrifugation (30,000 rpm for 16 hours); ^{125} I-labeled IL-1 α plus SS1 serum ($\blacksquare$); ^{125} I-labeled IL-1 α plus NHS1 serum ($\blacklozenge$); ^{125} I-labeled IL-1 α plus IgG-depleted SS1 serum ($\square$); and ^{125} I-labeled IL-1 α plus IgG-depleted NHS1 serum ($\diamond$). Note: the incorporation of ^{125} I-labeled IL-1 α into a higher molecular weight complex in NHS1 than in SS1 and the disappearance of complex formation when IgG was depleted in SS1 but not in NHS1.

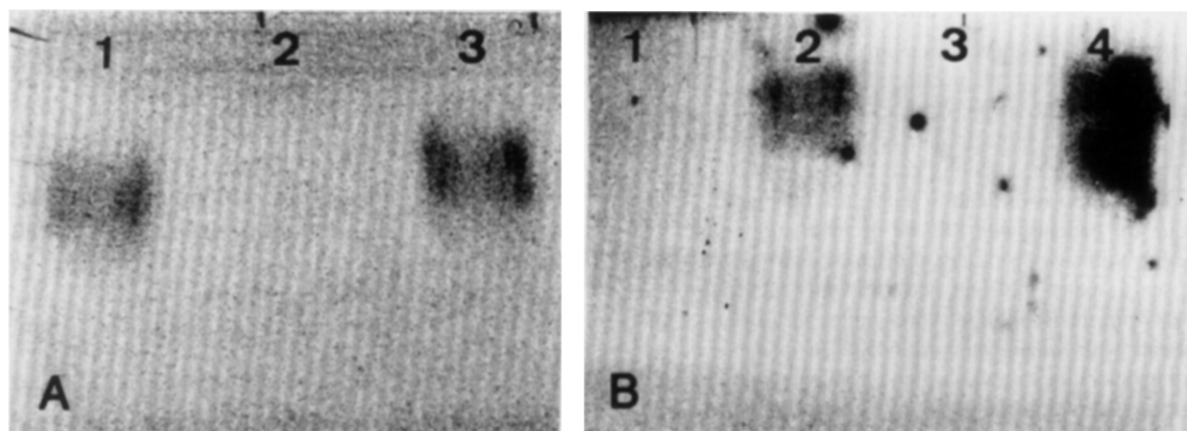


FIG. 4. Ligand blotting of SS1 and NHS1. Sera were run on 5% PAGE gels, transferred to nitrocellulose sheet, and then incubated with 0.132 nmol/L of ^{125} I-labeled IL-1 α and radioautographed. **A**, SS1 serum. Lane 1, binding of ^{125} I-labeled IL-1 α at IgG position, as determined by immunoblotting on separate sheet; lane 2, inhibition of this binding by 100-fold excess unlabeled IL-1 α ; lane 3, binding of ^{125} I-labeled IL-1 α that is not inhibited by the presence of 100-fold excess unlabeled IL-1 β . **B**, Lane 1, absence of binding of ^{125} I-labeled IL-1 α to NHS2 serum (negative control serum); lane 2, positive binding of ^{125} I-labeled IL-1 α at IgG position to SS1 serum; lane 3, absence of binding of ^{125} I-labeled IL-1 α to SS1 serum after IgG depletion; lane 4, binding of ^{125} I-labeled IL-1 α to NHS1 serum (see text and Fig. 3).

of SS1 and NHS1 sera and transfer onto nitrocellulose sheet, radiolabeled IL-1 α was revealed to bind at the position of IgG in both sera. The band of IL-1 α was wider with NHS1 than with SS1. However, the binding of SS1 was specific, since the radioactive bands disappeared by the addition of a 100-fold excess of

cold IL-1 α but not by the addition of a 100-fold excess of cold IL-1 β .

To characterize further the IL-1 α BF, the SS1- and NHS1-positive sera were tested by ligand binding to affinity-immobilized serum IgG; >90% of the radioactivity was retained in the column. Radioactivity

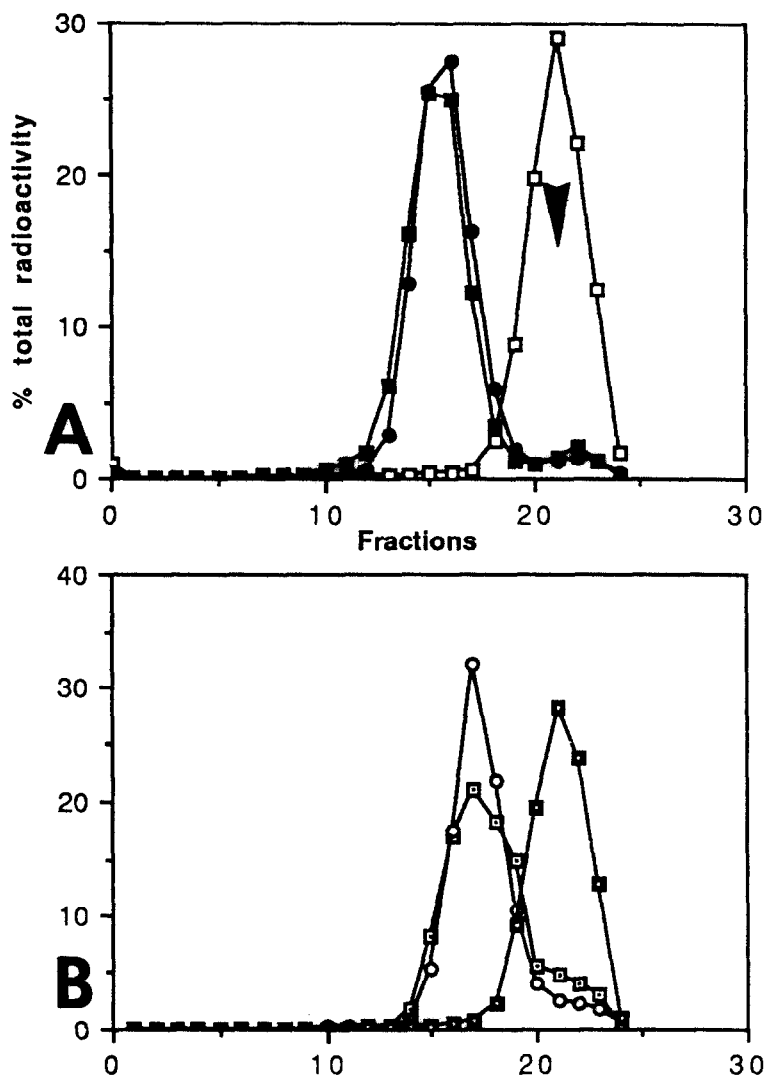


FIG. 5. Sucrose density-gradient centrifugation of ¹²⁵I-labeled IL-1 α bound to purified IgG and to the corresponding F(ab)₂ fragments (centrifugation at 30,000 rpm for 16 hours). **A**, IgG purified from two sera with IL-1 α BF SS1 IgG (●), NHS1 IgG (■), and NHS2 IgG (□) from a serum previously found to be devoid of IL-1 α BF. **B**, F(ab)₂ fragments obtained from SS1 IgG (○), from NHS1 IgG (□), and from NHS2 IgG (■). Arrow indicates the sedimentation of free ¹²⁵I-labeled IL-1 α .

eluted with an acidic elution buffer fell in the same fractions as IgG, suggesting that ¹²⁵I-labeled IL-1 α bound to the column via IgG. When a serum devoid of IL-1 α BF activity (subsequently referred to as NHS2), as assessed by the sucrose density-gradient assay, was used, IL-1 α did not bind to the protein G-Sepharose column (data not presented).

Protein G-purified IgG from both SS1 and NHS1 sera were able to displace ¹²⁵I-labeled IL-1 α in the sucrose density-gradient assay, whereas IgG from NHS2 serum did not (Fig. 5, A). To demonstrate that IL-1 α binding activity was due to the antigen-binding part of the IgG molecule, the experiments were per-

formed with F(ab)₂ fragments. Similar results were observed (Fig. 5, B).

To test whether IgG purified from sera having no detectable IL-1 α BF could inhibit the binding capacity of sera positive for IL-1 α BF in the sucrose density-gradient assay, 90 μ l of 10 pooled sera negative for IL-1 α BF were mixed with 10 μ l of IgG isolated from NHS1 serum before incubation with ¹²⁵I-labeled IL-1 α . No such inhibitory activity could be detected, suggesting that the negative sera tested did not contain factors that block IL-1 α BF, such as anti-idiotypic Ab (data not presented).

The affinity of the IL-1 α anti-Abs was studied by

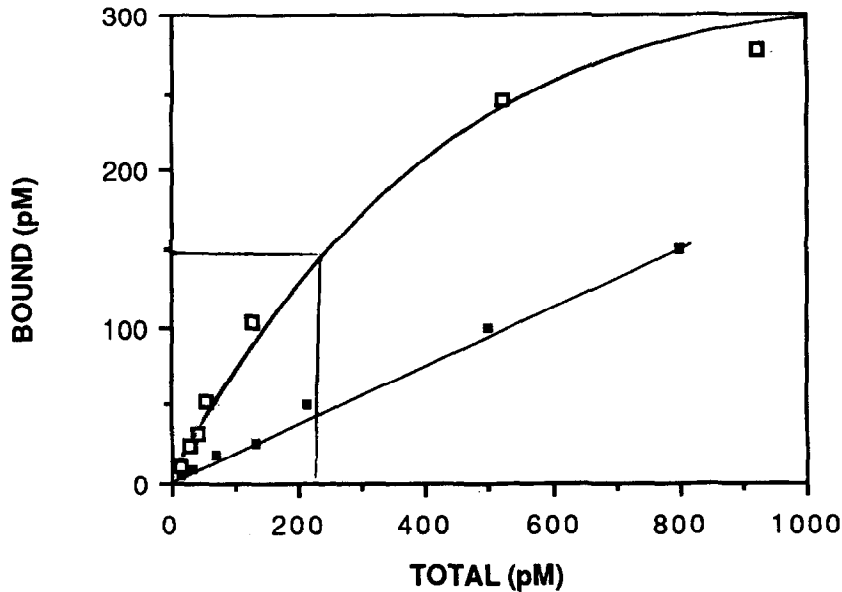


FIG. 6. K_m for ^{125}I -labeled IL-1 α of SS1 serum. One hundred microliters of serum were incubated with 0.010 to 1 nmol/L of ^{125}I -labeled IL-1 α (□). K_m was calculated at 208 pM, taking into account the specific binding only. The nonspecific binding was determined from the residual binding of ^{125}I -labeled IL-1 α in the presence of 100-fold excess of cold IL-1 α (■). Determinations made in triplicate (variation <9%).

TABLE I. Longitudinal study of IL-1 α BF in a patient with SS

Clinical and laboratory data										IL-1 α BF	
Date of serum sampling (mo/yr)	Fev	Urt	Bp	Sr	IgM (gm/L)	IgG (gm/L)	Pred (mg/kg/day)	PEG*	K_m (pM)	Concentration of anti-IL-1 α IgG	
										ng/ml	ng/mg total IgG
12/87	38°	+++	++	70	4.8	12	No	25	ND	71.96	5.9
3/87	38°	+	+	35	4.9	8.7	30	27	200	84.80	9.7
8/88	37°	±	±	14	8.3	9.1	10†	12	ND	34.76	3.8
2/89	37°	±	±	18	7.8	10.6	10†	20	208	59.96	5.6
10/89	37°	±	±	10	9.8	11.5	10†	22	ND	64.38	5.5

Fev, Fever; *urt*, urticaria; *bp*, bone pain; *sr*, erythrocyte sedimentation rate; *pred*, prednisone; *PEG*, titer of IL-1 α BF in the PEG precipitation assay; *ND*, not determined.

*Percent of precipitation by serum diluted 1/20 to increase sensitivity since precipitation in undiluted serum was 60% in all samples.

†Prednisone associated with ibuprofen, 1.2 gm/day.

PEG precipitation (Fig. 6). In a patient with SS (SS1 serum), the apparent K_m was 0.2×10^{-10} mol/L and was similar in two different sera obtained during a 3-year period (Table I). These tests permitted the estimation of the concentration of specific anti-IL-1 α auto-Ab (Table I). A mean antigen/Ab ratio of 1 was chosen for our estimation. The results demonstrate that the concentration of the auto-Ab was in the nanogram range (Table I) and represented only an extremely small fraction of total IgG ($<1/10^5$). The titer

of the Ab did not significantly change during a 3-year period.

Inhibition of ^{125}I -labeled IL-1 α binding to its cell-surface receptor in vitro

For these experiments nine sera were selected on the basis of the presence or absence of IL-1 α BF in the PEG precipitation assay. These included IL-1 α BF-positive sera from three patients with SS (including SS1 serum), IL-1 α BF-positive sera from three

TABLE II. Inhibition of IL-1 α binding to EL4-6.1 receptors by sera with IL-1 α BF

IL-1 α BF			IL-1 receptor binding on EL4-6.1 cells (% inhibition)								
Patients' sera	Peg assay* (%)	Ig isotype	Serum			IgG depleted serum			Purified IgG		
			1/16	1/32	1/64	1/16	1/32	1/64	1/16	1/32	1/64
SS1	60	IgG4	98	81	58	0	0	0	104	90	65
SS2	24	ND	9	0	0	ND			ND		
SS3	14	ND	0	0	0	ND			ND		
NHS1	66	IgG4 + IgA	96	74	47	0	0	0	87	71	46
NHS3	48	IgG4	0	0	0	ND			ND		
NHS4	30	ND	0	0	0	ND			ND		
NHS2	<10	—	11	0	0	0	0	0	0	0	0
NHS5	<10	—	0	0	0	ND			ND		
NHS6	<10	—	0	0	0	ND			ND		

Ig, immunoglobulin; ND, not determined.

In a first experiment, nine sera (diluted 1/16, 1/32, and 1/64) with different IL-1 α binding activities in the PEG assay were preincubated for 1 hour at 37° C with ¹²⁵I-labeled IL-1 α before addition to EL4-6.1 cells. Sera SS1 to 3 are from patients with SS and other sera from normal subjects. In a second experiment, purified IgG and corresponding IgG-depleted sera were incubated in the same way. Incubation on cells were done in duplicate (variation <5%).

*Percent of ¹²⁵I-labeled IL-1 α precipitated.

TABLE III. Organ distribution of ¹²⁵I-labeled IL-1 α 1 hour after intravenous injection in rats

Experiment No.	Plasma* (ml)	Kidney†	Muscle†	Skin†	Spleen†	Liver†	Lung†
1. IL-1 α	0.64	10.2	0.110	0.380	0.69	0.24	0.55
2. IL-1 α	0.44	13.4	0.110	0.355	0.61	0.21	0.44
3. IL-1 α /IgG-NHS2	0.48	9.5	0.120	0.380	0.79	0.30	0.56
4. IL-1 α /IgG-SS1	2.09	6.48	0.059	0.230	0.60	1.37	0.73
5. IL-1 α /IgG-NHS1	5.34	2.32	0.070	0.170	0.65	1.30	1.97
6. IL-1 α /IgG‡	4.07	3.80	0.052	0.120	0.65	0.79	0.98
	$p = 0.025§$	$p = 0.017$	$p = 0.001$	$p = 0.004$	NS	$p = 0.009$	NS

NS, No significant difference.

Measured in three tissue samples for each organ (variation <8%).

*Percent of total radioactivity injected per milliliter of plasma.

†Percent of total radioactivity injected per gram of wet tissue.

‡IgG obtained from multiple donors containing anti-IL-1 α auto-Abs.

§Significant differences in tissue distribution of free (experiments 1, 2, and 3) versus complexed (experiments 4, 5, and 6) IL-1 α determined by Student's paired t test.

normal human subjects (including NHS1 serum), and IL-1 α BF-negative sera from three normal subjects (including NHS2 serum). Two of the six IL-1 α BF-positive sera inhibited the binding of ¹²⁵I-labeled IL-1 α to EL4-6.1 cells, whereas four sera did not (Table II). Identical results were obtained with the purified IgG of the two inhibitory sera, whereas after IgG-depletion sera, these sera no longer inhibited the binding. None of the three IL-1 α BF-negative sera inhibited significantly the binding of ¹²⁵I-labeled IL-1 α on EL4-6.1 cells. It is noteworthy that the NHS1 serum was found to contain additionally IL-1 α BF of the IgA isotype (Fig. 7, serum No. 7); inhibition of receptor binding in this case was due to IgG and not

IgA, since IgG-depleted serum did not inhibit the binding (Table II).

IL-1 α clearance studies in rat

To determine if the auto-Abs modified the distribution of IL-1 α in body compartments, two rats were injected with ¹²⁵I-labeled IL-1 α alone, one rat with the ¹²⁵I-labeled IL-1 α plus IgG from SS1 serum, one rat with the ¹²⁵I-labeled IL-1 α plus IgG from NHS1 serum (both having anti-IL-1 α Ab), and one rat with ¹²⁵I-labeled IL-1 α plus IgG from NHS2 serum (IL-1 α BF negative). The plasma levels of ¹²⁵I-labeled IL-1 α were followed for more than 1 hour (Fig. 8). ¹²⁵I-labeled IL-1 α was cleared by 90% after 1 hour. When

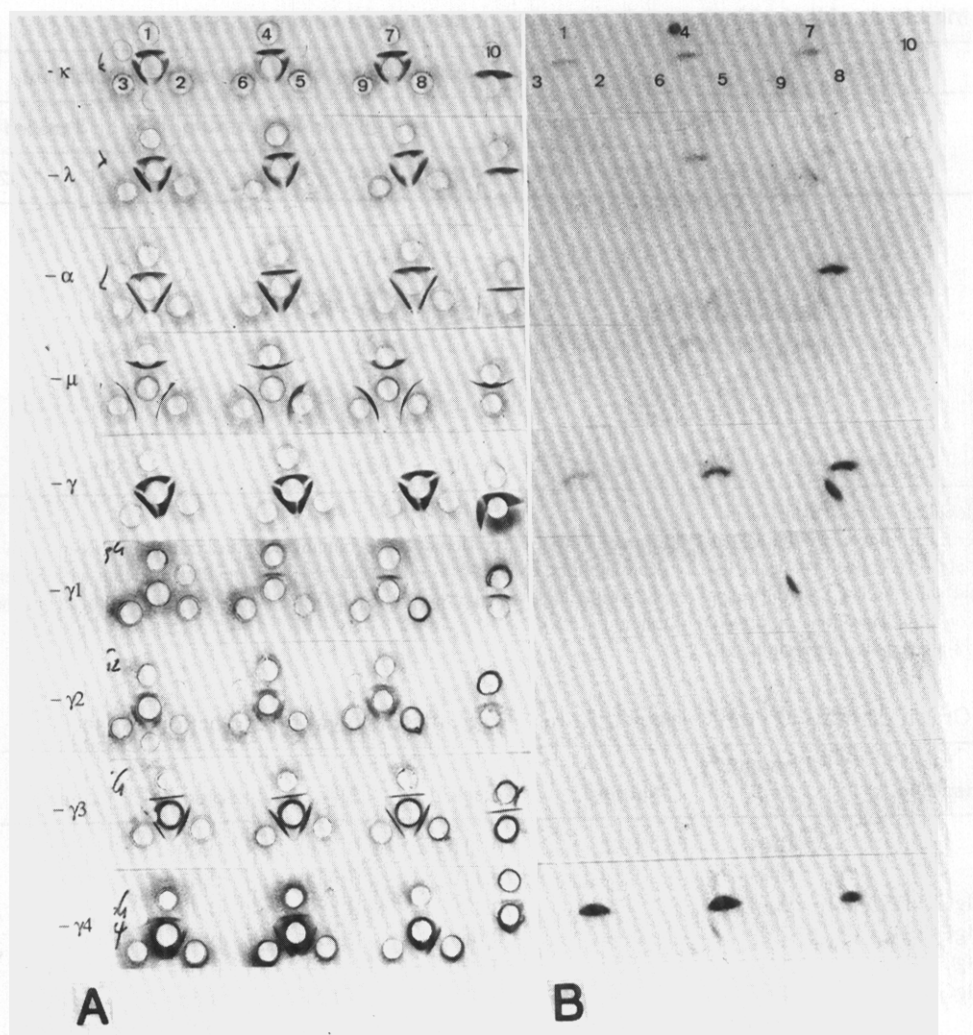


FIG. 7. Immunoglobulin classes and subclasses with ^{125}I -labeled IL-1 α binding activity revealed by double immunodiffusion-autoradiography technique. Ten positive sera for IL-1 α BF (nos. 1 to 10 demonstrated in the holes of the first row) tested with antisera to human κ and λ light immunoglobulin chains and α , μ , γ , and $\gamma 1$ to 4 heavy immunoglobulin chain subclasses. **A**, Coomassie brilliant blue protein staining. **B**, Autoradiography of **A**. The radioactive lines in **B** indicate immunoglobulin-IL-1 α complexes (see Table V for the summary of these observations).

^{125}I -labeled IL-1 α was complexed to auto-Abs, the elimination was slower (50% elimination in 25 minutes and >60 minutes, versus 7 ± 2 minutes in the control subjects). Other animals injected with ^{125}I -labeled IL-1 α plus an IgG preparation obtained from multiple donor, which also contained specific auto-Abs, provided similar results (50% elimination in >60 minutes). No ^{125}I -labeled IL-1 α was detected on circulating cells at any time point tested. The modification of the ^{125}I -labeled IL-1 α distribution by auto-Abs in other body compartments was demonstrated by measuring radioactivity of tissue samples (Table III). There was significantly less accumulation of radioactivity in the kidney of animals injected with ^{125}I -labeled IL-1 α -IgG complexes than with free ^{125}I -

labeled IL-1 α . The accumulation of free ^{125}I -labeled IL-1 α might be explained by filtration through the glomeruli, since IL-1 α has a small molecular weight.¹² The radioactivity found in skin and muscles was diminished by approximately 50% in the animals injected with ^{125}I -labeled IL-1 α -IgG, compared to animals injected with free ^{125}I -labeled IL-1 α , indicating clearly poor distribution (or uptake) of complexed ^{125}I -labeled IL-1 α in these organs. Although the splenic concentration of radioactivity was similar in all animals, specific accumulation was observed only in rats injected with free ^{125}I -labeled IL-1 α , as indicated by a higher ^{125}I -labeled IL-1 α content in spleen than in plasma. Finally, liver and lung of animals injected with complexed IL-1 α demonstrated more radioactiv-

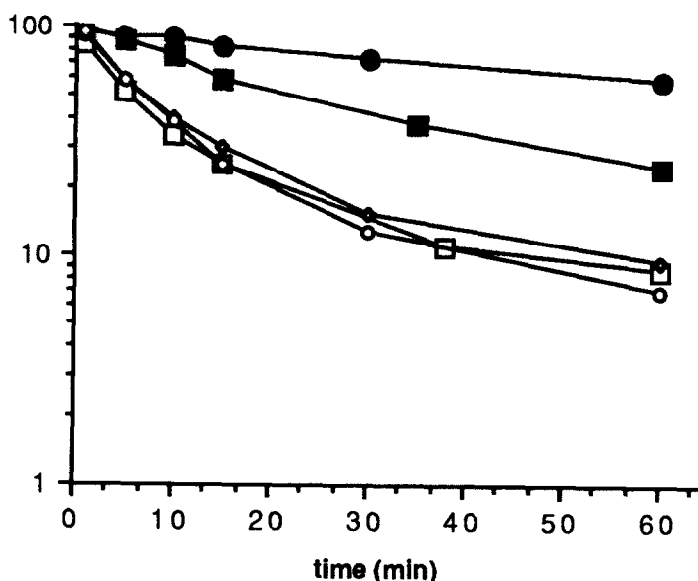


FIG. 8. ^{125}I -labeled IL-1 α clearance study in rats. Percentage of protein-bound radioactivity in rat blood after intravenous injection of either ^{125}I -labeled IL-1 α (400,000 cpm) alone in two rats ($\diamond$), ^{125}I -labeled IL-1 α preincubated with 20 mg of IgG purified from two sera containing IL-1 α BF (SS1 serum ($\bullet$) and NHS1 serum ($\blacksquare$), or with 20 mg of IgG from NHS2 serum devoid of IL-1 α BF ($\square$). Each determination was made in duplicate (variation <5%).

ity than the animals injected with free ^{125}I -labeled IL-1 α . However, there was no specific uptake of radioactivity in these organs as documented by the absence of an organ-plasma gradient. Thus, results might then be due to the presence of blood remaining in liver and lung.

Frequency and type of IL-1 α BF in 271 subjects

To define the incidence of IL-1 α BF in normal individuals and various diseases, we screened a total of 271 patients (Table IV) with the PEG precipitation assay. It was considered that only a precipitation exceeding 10% of the total radioactivity after correction for nonspecific precipitation (see METHODS section) indicated significant IL-1 α BF.

Eighteen percent of sera from normal control subjects were positive for IL-1 α BF in the PEG assay (Table IV). The frequency of IL-1 α BF-positive sera was not different in any of the groups tested except in the group with SS (66%; $p < 0.005$).

The affinity of IL-1 α BF was similar in four sera of patients with SS and seven other positive sera (K_m were between 0.1 and 0.3×10^{-9} mol/L).

Isotype distribution was tested by double immunodiffusion autoradiography in 27 sera. Sixteen of 19 sera with >40% precipitation in the PEG assay were positive in the double immunodiffusion assay. All eight other sera tested were negative (Table V; Fig. 7). Since six of these eight sera were positive in the

PEG binding assay, the sensitivity of the double immunodiffusion was low. However, some interesting observations could be made.

First, auto-Abs were not restricted to the IgG class; five auto-Abs were positive for IgA, but none for IgM. This observation explains why two IL1 α BF were observed in NHS1 serum (Figs. 3 and 4), which indeed contained IgG and IgA auto-Abs.

Second, there was a clear predominance of IgG subclass 4.

Third, reactivity with both light chains was present in only four cases. This finding might indicate that the auto-Abs are produced by a very limited number of clones, although no firm conclusions should be drawn because of the poor sensitivity of the assay.

Finally, only IgG and IgG4 reactivity were detected in patients with SS.

We tested 37 sera (13 from the group with SS and 24 sera from the other groups) for IL-1 β BF and TNF- α BF by sucrose gradient assay. Only one serum was found to be positive for IL-1 β and TNF- α . This serum was from a patient with SS (not SS1 serum) who also had a positive test for IL-1 α BF.

DISCUSSION

The possibility that human sera contain Abs against cytokines, such as IL-1 α ,^{4,5} raises new dimensions in the understanding of cytokine function in vivo. The significance of these Abs in regulating cytokine activity needs to be considered analogously to that of

TABLE IV. Incidence of IL-1 α BF(s) as analyzed by PEG precipitation assay in the serum of 271 patients

	N	IL-1 α BF positive sera*	
		N	%
Normal subjects	93	17	18
SS	9	6†	66
Control subjects			
Inflam dermatoses with monoclonal Ig	9	2	22
Monoclonal IgM	2	0	0
Inflam dermatoses without monoclonal Ig	90	14	15
Chronic or intermittent pyrexia‡	28	4	14
Kidney graft	10	1	10
Elderly patients	30	6	20

Inflam, Inflammatory; *Ig*, immunoglobulin.

*Ten percent or more ¹²⁵I-labeled IL-1 α precipitated (see text).

†Fischer's exact test corrected for continuity for number of positive sera, $p < 0.005$, found in normal subjects versus group with SS. Compared to normal subjects, the other groups did not differ.

‡Systemic lupus erythematosus, 3/15; cryoglobulinaemia, 0/7; AIDS, 1/6.

the native IL-1 inhibitors.¹³ In this article, we present data on three aspects of anti-IL-1 α auto-Abs: immunochemical characterization, functional properties, and clinical correlations.

Immunochemical characterization

A sucrose density-gradient assay reproducibly identified IL-1 α BF. Attempts to determine the nature of IL-1 α BF demonstrated that the binding activity is due to Abs (auto-Abs) of the IgG class. Evidence for this finding was demonstrated by (1) IgG affinity purification and depletion, (2) ligand blotting, (3) ligand binding to immobilized IgG, (4) immunodiffusion autoradiography, and (5) binding activity of IgG was associated with the F(ab)₂ fragment. IL-1 α BF of the IgA isotype was also detected by the immunodiffusion-autoradiography technique. The finding of auto-Abs restricted to IgA and IgG4 is not common¹⁴; auto-Abs are usually IgM and predominantly IgG1 and IgG3.¹⁵ Convincing data were obtained to demonstrate that most Abs to IL-1 α in the IgG fraction were of the IgG4 isotype. This finding is interesting from a number of points of view.

First, Abs to protein antigens are usually of the IgG1 and IgG3 isotype,¹⁵ but exceptions exist in which IgG4 predominates.¹⁶ This is particularly evident when booster immunizations and prolonged antigenic chal-

lenge are used.¹⁶ Our findings therefore suggest that IL-1 α BF arise as a consequence of persistent stimulation. This finding is also supported by the fact that these auto-Abs were of high affinity.

Second, it is important to note that IgG4 only constitutes 4% to 5% of the IgG in serum; production of anti-IL-1 α auto-Abs restricted to this isotype may be of benefit to the host. Unlike Abs of IgG1 and IgG3, which activate complement, and, in this way, may mediate pathologic events, IgG4 Abs do not activate complement.

Third, IgG4 Abs produce small nonprecipitating immune complexes¹⁷ and therefore, rather than clear the cytokine, may act as an important transport mechanism. We, in fact, present evidence to this effect. Injection of auto-Abs-treated IL-1 α into rats increased its half-life in the circulation.

The concentration of the IL-1 α IgG auto-Abs was estimated to be in the nanogram range and represented only an extremely small fraction of total IgG ($<1/10^{-5}$). All these observations should be considered when the potential functional role of these auto-Abs are being analyzed.

Functional properties

Little is known about the biologic activity of IL-1 α auto-Abs. Suzuki et al.¹⁸ have demonstrated that sera from patients with rheumatoid arthritis inhibited the IL-1 α -induced endothelial cell adherence of neutrophils; the inhibitory activity was present in the IgG fraction. Our results support and extend these findings by demonstrating that IL-1 α IgG auto-Abs do interfere with IL-1 α in two biologic systems.

First, auto-Abs inhibited its binding of ¹²⁵I-labeled IL-1 α to cell-surface receptors of the murine thymoma EL4-6.1 cells. This finding indicates that epitopes recognized by some of these auto-Abs may interfere with a domain of IL-1 α necessary for binding to the cells, and confirms more recent observations by Suzuki et al.¹⁹ However, not all IL-1 α BF-positive sera were found to block ¹²⁵I-labeled IL-1 α binding to the IL-1 cellular receptor, even though they had IL-1 α BF of the same class (IgG), subclass (IgG4) affinity, and serum concentration of the blocking sera; this finding suggests that these sera recognize epitopes on the IL-1 α molecule that are not involved in binding to the IL-1 α receptor of EL4-6.1 cells. This finding, having Abs that do not block activity but might increase the half-life of IL-1 α , may be of major significance.

Second, we found that IL-1 α auto-Abs altered significantly the plasma clearance of ¹²⁵I-labeled IL-1 α , as well as its tissue distribution, when these Abs were injected into rats, in particular, less IL-1 α was recov-

TABLE V. Class and subclass distribution of the anti-IL-1 α auto-Abs in 27 sera examined by the double immunodiffusion-autoradiography technique

% ¹²⁵ I-IL-1 α precipitated in the PEG assay	Radioactive bands after reaction with antihuman immunoglobulin chains										
	No. of negative sera	No. of positive sera									
		κ	λ	κ and λ	γ	1	2	3	4	α	μ
>40 (n = 19)	3	8	5	4	12	1	0	1	9	5	0
<40 (n = 8*)	8	—	—	—	—	—	—	—	—	—	—

*Six sera were positive (PEG precipitation between 10% and 40%); two sera were negative (PEG <10%).

ered in skin and muscle. This indicates that the Ab IL-1 α complex does not dissociate rapidly in vivo and supports the hypothesis that auto-Abs to IL-1 α could modulate systemic as well as local effects of IL-1 α .⁴ Fomsgaard et al.²⁰ speculated that circulating TNF- α would be cleared more rapidly from the blood by binding to anti-TNF- α Abs. Our data on IL-1 α do not support this concept.

It is difficult to extrapolate from these experimental observations whether prolonged serum half-life would ultimately result in inhibition or potentiation of the IL-1 α effects in humans. However, a modulating effect on IL-1 α actions appears likely, since IgG and IgA have a wide tissue distribution, also in extravascular compartments, and since IgG accumulate at inflammatory sites.²¹ By changing the half-life of IL-1 α in body compartments and by modulating the binding of IL-1 α to its receptor, auto-Abs to IL-1 α might increase or diminish, prolong or shorten biologic effects of this important proinflammatory cytokine. Abs to hormones have been described to enhance hormone activity.²²

Clinical correlations

The screening for IL-1 α auto-Abs in 271 sera was made by a PEG precipitation assay. The overall incidence of IL-1 α BF_s was 18%. Since incidence in elderly patients (older than 80 years) was not increased, the possibility that these are age related is excluded. The frequency, affinity, class, and subclass distribution of auto-Abs to IL-1 α was not different in normal control subjects and patients with various febrile and inflammatory diseases.

The only group that differed from normal subjects was the group with SS. Although this group represents the largest series of cases with SS so far reported, the number is small, and the results may still be proved to be in error; however, the frequency of IL-1 α BF was not increased in several control groups of patients chosen to closely mimic the signs and symptoms of this syndrome. SS is characterized by chronic urti-

caria, bone pain, fever, and monoclonal IgM,⁸ which appeared compatible with a dysregulation of a cytokine.

Since anti-IL-1 α IgG was found in normal subjects, it cannot be directly implicated in the symptoms of SS. This is in agreement with the results from the 3-year follow-up study of one patient with this syndrome. There were no changes of either titer, binding activity, or affinity of the IL-1 α auto-Abs, even though periods of active disease with fever, bone pain, and urticaria followed periods of clinical remission with steroid therapy.

Increased frequency of IL-1 α auto-Abs in SS might rather indirectly reflect some alteration in the Ab-regulated network of cytokines in these patients. Preliminary studies have detected IgM auto-Abs to IL-1 α by immunoblot analysis in some patients with this syndrome, suggesting that the monoclonal IgM was implicated²; this finding could not be confirmed at the present time with the other techniques used in the present study, possibly because such methods were not appropriate to identify IgM auto-Abs. Works are in progress to elucidate this point, since the monoclonal IgM might well interfere with the IL-1 α auto-Abs network in these patients.

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REFERENCES

1. Schnitzler L, Hurez D, Verret JL. Urticaire chronique, ostéocondensation, macroglobulinémie. Cas princeps. Etude sur 20 ans. *Ann Dermatol Venerol* 1989;116:547-50.
2. Didierjean L, Saurat JH. Schnitzler syndrome (urticaria and macroglobulinemia): serum IgM immunoreactivity against interleukin-1 (IL-1) as shown by immunoblotting [Abstract]. *J Invest Dermatol* 1990;94:393.
3. Shelley WB, Shelley ED. Delayed pressure urticaria syndrome:

- a clinical expression of interleukin-1. *Acta Derm Venereol* (Stockh) 1987;67:438-59.
4. Bendtzen K, Svenson M, Fomsgaard A, Poulsen LK. Native inhibitors (autoantibodies) of IL-1 α and TNF. *Immunol Today* 1989;10:222.
 5. Svenson M, Poulsen LK, Fomsgaard A, Bendtzen K. IgG autoantibodies against Interleukin-1 α in sera of normal individuals. *Scand J Immunol* 1989;29:489-92.
 6. Seckinger P, Williamson K, Balavoine JF, et al. A urine inhibitor of interleukin-1 activity affects both interleukin-1 α and 1 β but not tumor necrosis factor α . *J Immunol* 1987;139:1541-5.
 7. Schifferli JA, Steiger G, Hauptmann G, Spaeth PJ, Sjöholm AG. Formation of soluble immune complexes by complement in the sera of patients with various hypocomplementemic states. *J Clin Invest* 1985;76:2127-33.
 8. Janier M, Bonvalet D, Blanc MF, et al. Chronic urticaria and macroglobulinemia (Schnitzler's syndrome): report of two cases. *J Am Acad Dermatol* 1989;20:206-11.
 9. Pujol RM, Barnadas MA, Brunet S, de Moragas JM. Urticarial dermatosis associated with Waldenström's macroglobulinemia. *J Am Acad Dermatol* 1989;20:855-7.
 10. Doutre MS, Beylot C. Chronic urticaria and monoclonal IgM: treatment with ibuprofen. *J Am Acad Dermatol* 1990;22:143-4.
 11. Bonnetblanc JM, Drouet M, Laplaud P, Bedane C, Bernard P. Urticaria with macroglobulinaemia: disease activity associate alterations in immunoglobulins profile and bone marrow hypodiploidy. *Dermatologica* 1990;181:41-3.
 12. Maak T, Johnson V, Kan ST, Figueiredo J, Sigulem D. Renal filtration, transport, and metabolism of low-molecular-weight proteins: a review. *Kidney Int* 1979;16:251-70.
 13. Dayer JM, Seckinger P. Natural inhibitors and antagonists of interleukin-1. In: Bomford R, Henderson B, eds. *Interleukin-1, inflammation and disease*. Amsterdam: Elsevier Science, 1989:283-302.
 14. Flotte TJ, Baird LG. Immunoglobulin light and heavy chain isotypes in skin diseases: restricted distribution in bullous pemphigoid and linear IgA bullous dermatosis. *J Immunol* 1986;136:491-6.
 15. Young WJ, Cohen P, Eisenberg RA. Distribution of IgG subclasses among human autoantibodies to Sm, RNP, dsDNA, SS-B, and IgG rheumatoid factor. *Monogr Allergy* 1988;23:41-56.
 16. Ferrante A, Beard LJ, Feldman RG. IgG subclass distribution of antibodies to bacterial and viral antigens. *Pediatr Infect Dis J* 1990;9(suppl 8):516-24.
 17. Van der Zee JS, van Swieten P, Aalberse RC. Serologic aspects of IgG4 antibodies. II. IgG4 antibodies form small, nonprecipitating immune complexes due to functional monovalency. *J Immunol* 1986;137:3566-71.
 18. Suzuki H, Akama T, Okane M, et al. Interleukin-1-inhibitory IgG in sera from some patients with rheumatoid arthritis. *Arthritis Rheum* 1989;32:1528-38.
 19. Suzuki H, Kamimura J, Ayabe T, Kashiwagi H. Demonstration of neutralizing autoantibodies against IL-1 α in sera from patients with rheumatoid arthritis. *J Immunol* 1990;145:2140-6.
 20. Fomsgaard A, Svenson M, Bendtzen K. Autoantibodies to tumour necrosis factor α in healthy humans and patients with inflammatory diseases and gram-negative bacterial infections. *Scand J Immunol* 1989;30:219-23.
 21. Rubin RH, Fischman AJ, Callahan RJ, et al. ¹¹¹In-labeled non-specific immunoglobulin scanning in the detection of focal infection. *N Engl J Med* 1989;321:935-40.
 22. Aston R, Cowden WB, Ada GL. Antibody-mediated enhancement of hormone activity. *Mol Immunol* 1989;26:435-46.

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