Inhibition of natural killer cell activity by serum from patients with systemic lupus erythematosus: roles of disease activity and serum interferon

STEVEN R. YTTERBERG AND THOMAS J. SCHNITZER

From the Rackham Arthritis Research Unit, Department of Internal Medicine, University of Michigan, USA

SUMMARY Among their immunological alterations patients with systemic lupus erythematosus (SLE) have been shown to have diminished natural killer (NK) cell activity. This abnormality is at least in part related to humoral factors, as sera from patients with SLE can inhibit the NK activity of peripheral blood mononuclear cells from normal individuals. The present study extends these findings to demonstrate that the inhibitory ability of sera from patients with SLE varies with disease activity. Furthermore, sera from patients with active SLE containing interferon (IFN), a potent stimulator of NK activity, were equally or more inhibitory than sera which did not contain IFN. Thus the factors in SLE sera which can inhibit NK function vary with disease activity and cannot be overcome by IFN present in these sera.

Natural killer (NK) cell activity is a cell-mediated cytotoxic function expressed by peripheral blood mononuclear cells of most normal individuals.¹ Although the precise role of NK activity *in vivo* is debated, current evidence suggests that it may be important for a variety of normal functions, including inhibition of tumour growth or spread, control of certain viral infections, and immunoregulation.^{1,2}

Diminished NK cytotoxicity has been described in several disease states, including systemic lupus erythematosus (SLE),³⁻⁶ but the significance of this finding for disease expression is not known. Recently it has been shown that the diminished NK cell function in patients with SLE may be due in part to circulating inhibitory factors, as sera from patients with SLE can inhibit the NK activity of peripheral blood mononuclear cells from normal individuals.^{7,8} Interestingly, sera from patients with active SLE also often contain interferon (IFN),^{9,10} a potent stimulator of NK activity.^{11,12} To assess the relationship between inhibition of NK activity by sera from patients with SLE and clinical disease activity, as well as the occurrence of IFN in these sera, the present study was undertaken. The results indicate that the

ability of sera from patients with SLE to inhibit NK cytotoxicity of normal mononuclear cells varies with disease activity and is independent of the presence of IFN in the sera.

Materials and methods

Serum samples. Serum samples were obtained from 20 patients with SLE who met the preliminary classification criteria of the ARA¹³ and were compared with normal sera from 9 healthy individuals. Serum samples were collected aseptically, stored at -20°C until use, and heat-inactivated at 56°C for 30 minutes.

Clinical data. Patients' data were gathered by chart review. Patients were considered to have active disease if there was clinical evidence of disease activity involving any organ system and the anti-DNA binding activity was greater than 40.0% (Farr technique). Stable renal involvement was not considered a sign of active disease. Patients lacking these criteria were deemed to be clinically inactive.

Effector cells. Normal mononuclear cells (MN) and lymphocyte-enriched populations obtained from peripheral blood of healthy volunteers, prepared as previously described,⁸ were used as effector cells. The lymphocyte-enriched populations contained more than 97% lymphocytes by morphology. Effec-

Accepted for publication 9 September 1983.
Correspondence to Thomas J. Schnitzer MD, Rackham Arthritis Research Unit, R4633, Kresge I, University of Michigan, Ann Arbor, Michigan 48109, USA.

tor cells were suspended at 10×10^6 cells/ml in RPMI-1640 medium supplemented with 50 μ g/ml gentamicin, 2 mM glutamine, and 10% newborn calf serum (RPMI-10%NCS) (RPMI = Roswell Park Memorial Institute). Viability of effector cells was assessed by their ability to exclude trypan blue. All effector cell populations were > 96% viable when assessed by trypan blue exclusion.

NK assay. Target cells for NK cytotoxicity were a human leukaemia tumour cell line, K562. The cells were labelled with ^{51}Cr ⁸ and resuspended in RPMI-10%NCS at 2×10^5 cells/ml. NK activity was measured in triplicate in a 4-hour chromium release assay as previously described.⁸ 50 μ l of each test serum was placed in a microtitre well, followed by 50 μ l of effector cells and 50 μ l of target cells, yielding an effector : target cell ratio of 50:1. Percentage

cytotoxicity was calculated as percentage specific ^{51}Cr release = (cpm of experimental sample - cpm spontaneous release)/(total cpm added per well - cpm spontaneous release) $\times$ 100. Spontaneous release, measured in the presence of RPMI-10%NCS but without effector cells, was $10.3 \pm 0.4\%$. Control sera ($n=1-6$) were assayed for each individual experiment. Mean cytotoxicity in the presence of the control sera was calculated and the percentage inhibition for each serum sample calculated on the basis of this standard for the individual experiment. Data were compared for statistical significance by Welch's approximation of Student's *t* test.¹⁴ Grouped data are expressed as the mean $\pm$ SEM except as noted.

Interferon assay. A previously described,¹⁰ microtitre plaque-reduction assay for IFN was used,

Table 1 Clinical disease activity and therapy of patients studied

Patient	Inhibition of NK Activity (%) ^a		IFN ^b	DNA ^c	Clinical manifestations ^d	Therapy ^e	
	Mn	Lymph				Pred	Other
A. Patients with active disease							
1	55	ND ^f	+	45.8	Rash, alopecia	35	Plq
2	35	51	ND	54.4	Arth, CNS, leucopenia	15	ASA, Plq
3	24	48	—	48.4	Renal, thrombocytopenia	Pulse	—
4	47	ND	+	67.5	Renal	60	—
5	54	48	+	50.3	Arth, syst	—	Ibuprofen
6	30	14	—	86.5	Arth, rash, renal	—	—
7	49	30	ND	86.5	Arth, rash, MM, sero, syst	—	ASA
8	22	35	—	63.3	Renal	—	—
9	42	37	ND	51.8	Arth, rash, Ray, syst	5	ASA
10	2	—20	ND	40.0	Cutaneous vasculitis	40	ASA
11	40	24	+	92.3	Arth, rash, MM, renal	40	—
12	27	14	+	85.6	Rash, alopecia, CP, syst	30	Plq
13	50	53	+	77.9	Arth, rash, leucopenia	40	—
14	26	18	ND	56.9	CNS	20	—
15	16	ND	ND	89.5	Arth, rash, MM, leucopenia	—	—
16	—1	0	+	89.2	Arth, rash, syst	—	—
Mean	32	27					
±SEM	±4	±6					
B. Patients with inactive disease							
17	23	15	—	29.8	None	2	
18	—4	0	ND	13.2	Abd pain, none otherwise	12	
19	31	—4	—	28.0	None	11	ASA, Plq
20	26	3	—	26.4	Stable proteinuria	15	
21	—1	ND	ND	17.5	None	20	
Mean	±7	±4					

^a Percentage inhibition of NK activity of normal mononuclear effector cells toward K562 targets. Values are expressed as a percentage of the cytotoxicity observed in the presence of serum from normal donors, as described in 'Materials and methods.' MN = peripheral blood mononuclear cells as effectors. Lymph = lymphocyte-enriched populations as effectors.

^b Interferon (IFN) titres determined by a microtitre plaque-reduction technique with human amnion (WISH) cells as targets and vesicular stomatitis virus, Indiana serotype (VSV), as challenge virus. Titres > 8 units/ml considered positive.

^c Anti-DNA binding activity (%) as determined by the Farr assay.

^d Disease activity at the time of serum acquisition, as determined by chart review. Abbreviations used: Rash = cutaneous; Arth = arthritis; CNS = central nervous system involvement; Syst = fever and/or fatigue; MM = mucous membrane lesions; Sero = serositis; Ray = Raynaud's phenomenon; CP = pleuritic chest pain; abd pain = abdominal pain.

^e Drugs being used at the time serum samples were obtained. Abbreviations: Pred = prednisone dose in mg/d; ASA = aspirin; Plq = hydroxychloroquine.

^f Not done.

which is based on the ability of IFN to cause a linear dose-dependent cellular resistance to viral infection.

Results

INHIBITION OF NK ACTIVITY BY SERA FROM PATIENTS WITH SLE

The clinical disease activity, therapeutic regimen, and level of serum-mediated inhibition of NK activity of each patient studied are shown in Table 1. With unfractionated mononuclear cells from normal individuals as effectors, the mean inhibition of NK activity by sera from patients with active disease was $32 \pm 4\%$ versus $15 \pm 7\%$ by sera from patients with inactive disease (Fig. 1). These levels of inhibition are significantly different from each other ($p < 0.05$) and from results obtained with control sera from normal individuals ($p < 0.001$ and $p < 0.025$, respectively).

When the studies were repeated with lymphocyte-enriched populations from normal indi-

viduals as effectors, inhibition was observed to be $27 \pm 6\%$ and $4 \pm 4\%$ for patients with active and inactive disease, respectively (Fig. 2). Significant inhibition by sera from patients with active disease was observed in comparison with results with sera from both normal controls and patients with inactive disease ($p < 0.001$ and $p < 0.005$, respectively), while no difference between patients with inactive disease and controls was seen.

ROLE OF CORTICOSTEROIDS IN INHIBITION OF NK ACTIVITY BY SLE SERA

Of patients with active disease 8 were receiving corticosteroids at the time the serum samples were obtained, while 5 patients were not. Comparison of sera from these groups of patients showed no statistical difference in their ability to inhibit NK activity. With MN effectors inhibition was $35 \pm 5\%$ for the steroid-treated group versus $28 \pm 8\%$ for those not

Fig. 1 Inhibition of NK activity of unfractionated mononuclear cells from normal donors toward K562 targets. NK assays performed in the presence of heat-inactivated serum from patients with active SLE, patients with inactive SLE, or normal controls. The mean ± 1 standard deviation is shown.

Fig. 2 Inhibition of NK activity of lymphocyte-enriched mononuclear cell populations from normal donors toward K562 targets. NK assays performed in the presence of heat-inactivated serum from patients with active SLE, patients with inactive SLE, or normal controls. The mean ± 1 standard deviation is shown.

receiving steroids. With lymphocyte-enriched effectors the values were $28 \pm 9\%$ versus $25 \pm 8\%$. Thus the inhibition of NK activity by sera from patients with active SLE cannot be explained simply by the presence of corticosteroid in the serum samples.

ROLE OF SERUM INTERFERON

Several sera from patients with active disease only minimally inhibited NK activity. As IFN is a potent stimulator of NK activity and is known to be present in sera from patients with SLE, quantitation of IFN in several of these serum samples was undertaken to determine if its presence might explain these results. Of 11 sera assayed from patients with active disease IFN was found in 7. Of these, 6 inhibited MN-mediated NK activity. Indeed, the sera containing IFN were among the most inhibitory sera examined. Of serum samples from patients with active disease those containing IFN caused a mean inhibition of MN-mediated NK activity of $39 \pm 8\%$ compared with a mean inhibition of $20 \pm 5\%$ for sera lacking measurable IFN ($0.1 > p > 0.05$).

Discussion

Diminished NK cell activity has been reported in patients with SLE,³⁻⁶ but neither the mechanism responsible for, nor the significance of, this observation is known. Sera from patients with SLE have been shown to inhibit NK activity,^{7,8} suggesting a role for humoral factors in effecting this alteration. Other lines of evidence suggest that an intrinsic defect of NK cells from patients with SLE may also exist.¹⁵

The present study has shown that the ability of sera from patients with SLE to inhibit the NK activity of normal MN populations is correlated with the presence of clinically active disease. These data suggest that as patients become clinically active they produce a factor(s) which is able to inhibit NK cytotoxicity. In addition this study has shown that sera from patients with inactive SLE inhibit NK cytotoxicity mediated by unfractionated MN but not lymphocyte-enriched populations. The reasons for this observation are not known but may relate to effects of the sera from patients with SLE on monocytes present in the unfractionated preparations, with secondary inhibitory effects being exerted on the NK cells. In contrast, the sera from patients with active disease may be able to inhibit NK cells directly. Furthermore, inhibition of NK activity by sera from patients with active SLE was shown to be independent of the presence of corticosteroids in the sera.

As IFN is known both to be present in sera of patients with active SLE^{9,10} and to stimulate in a potent manner NK cell activity,^{11,12} an attempt was made to determine its role in the phenomenon under

study. Interestingly, sera from patients with active SLE containing IFN were found to inhibit NK activity as effectively as sera from active patients which did not contain IFN. Indeed, the sera containing IFN were slightly more inhibitory than those which did not contain IFN. One explanation for this finding is that the inhibitory factors present are potent enough to overcome any stimulatory effects exerted by the IFN in the sera. In support of this hypothesis, the NK activity of mice treated with IFN inducers has been shown to rise initially following the induction of IFN but subsequently to fall below normal levels.¹⁶ Among the potential mechanisms to explain this finding is the suggestion that soluble suppressors of NK activity might be released by chronic stimulation of NK cells by IFN. If such suppressors of NK activity were released into the circulation of patients with SLE subsequent to chronic IFN stimulation, they might be able to mediate the inhibition observed. Alternatively, the IFN in these serum samples may have lost its ability to stimulate NK activity. This possibility has not been directly tested but must be considered in view of the marked abnormalities of the IFN-NK system in patients with SLE.¹⁷

The nature of the factor(s) responsible for the inhibition of cytotoxicity demonstrated in this study is not known. Several factors are present in the sera of patients with SLE which are capable of altering NK activity including immune complexes, which, along with monomeric IgG, inhibit NK activity.^{18,19} In addition, many patients with SLE have antilymphocyte antibodies (ALA). Recent studies have demonstrated that SLE patients with ALA have lower NK activity than those without ALA⁵ and that SLE sera can contain antibodies to purified populations of large granular lymphocytes, the subset of peripheral blood cells which mediates most NK activity in humans.²⁰ Further investigations are necessary to determine the nature of the inhibitor of NK activity in SLE sera as well as the significance of diminished NK activity for expression and control of disease in these patients. Additionally, further characterisation of the IFN present in SLE sera is warranted to determine whether it has normal immunoregulatory capabilities.

This work was supported in part by Training Grant AM-07080 from the US Public Health Service and a grant from the Upjohn Company. Dr Schnitzer is a Senior Fellow of the Arthritis Foundation.

References

- 1 Herberman R B, Holden H T. Natural cell-mediated immunity. *Adv Cancer Res* 1978; **27**: 305-77.
- 2 Herberman R B, Ortaldo J R. Natural killer cells: their role in defenses against disease. *Science* 1981; **214**: 24-30.

- 3 Lang I, Benczur M, Varga M, Gergely P, Petranyi G. Antibody dependent and spontaneous lymphocyte mediated cytotoxicity in normal subjects, patients with SLE, and malignancies. *Acta Med Acad Sci Hung* 1977; **34**: 123-7.
- 4 Hoffman T. Natural killer function in systemic lupus erythematosus. *Arthritis Rheum* 1980; **23**: 30-5.
- 5 Goto M, Tanimoto K, Horiuchi Y. Natural cell mediated cytotoxicity in systemic lupus erythematosus. Suppression by antilymphocyte antibody. *Arthritis Rheum* 1980; **23**: 1274-81.
- 6 Kozlowski S H, Hirszen D J, Jackson E J. Comparison of natural killing with antibody dependent cell mediated cytotoxicity in patients with systemic lupus erythematosus. *J Rheumatol* 1982; **9**: 59-62.
- 7 Silverman S L, Cathcart E S. Natural killing in systemic lupus erythematosus: inhibitory effects of serum. *Clin Immunol Immunopathol* 1980; **17**: 219-26.
- 8 Ytterberg S R, Huard T K, Schnitzer T J. Effects of serum from patients with systemic lupus erythematosus on leukocyte cytotoxicity: modulation of NK activity and ADCC. *J Rheumatol* 1984; **10**: 906-12.
- 9 Hooks J J, Moutsopoulos H M, Geis S A, Stahl N I, Decker J L, Notkins A L. Immune interferon in the circulation of patients with autoimmune disease. *N Engl J Med* 1979; **301**: 5-8.
- 10 Ytterberg S R, Schnitzer T J. Serum interferon levels in patients with systemic lupus erythematosus. *Arthritis Rheum* 1982; **25**: 401-6.
- 11 Trinchieri G, Santoli D. Anti-viral activity induced by culturing lymphocytes with tumor-derived or virus-transformed cells. Enhancement of human natural killer cell activity by interferon and antagonistic inhibition of susceptibility of target cells to lysis. *J Exp Med* 1978; **147**: 1314-33.
- 12 Djeu J Y, Heinbaugh J A, Holden H T, Herberman R B. Augmentation of mouse natural killer cell activity by interferon and interferon inducers. *J Immunol* 1979; **122**: 175-81.
- 13 Cohen A S, Reynolds W E, Franklin E C, *et al.* Preliminary criteria for the classification of systemic lupus erythematosus. *Bull Rheum Dis* 1971; **21**: 643-8.
- 14 Remington R D, Schork M A. *Statistics with applications to the biologic health sciences*. Englewood Cliffs, New Jersey: Prentice-Hall, 1970: 221-3.
- 15 Katz P, Zaytoun A M, Lee J H, Jr, Panusch R S, Longley S. Abnormal natural killer cell activity in systemic lupus erythematosus: an intrinsic defect in the lytic event. *J Immunol* 1982; **129**: 1966-71.
- 16 Melder R J, Ho M. Modulation of natural killer cell activity in mice after interferon induction: depression of activity and depression of in vitro enhancement by interferon. *Infect Immun-*
ity 1982; **36**: 990-5.
- 17 Tsokos G C, Rook A H, Djeu J Y, Balow J E. Natural killer cells and interferon responses in patients with systemic lupus erythematosus. *Clin Exp Immunol* 1982; **50**: 239-45.
- 18 Kay H D. Differential effects of immune complexes on human natural and antibody-dependent cell-mediated cytotoxicity. In: Herberman R B, ed. *Natural cell-mediated immunity against tumors*. New York: Academic Press, 1980: 329-46.
- 19 Sulica A, Gherman M, Galatiuc C, Manciulea M, Herberman R B. Inhibition of human natural killer cell activity by cytophilic immunoglobulin G. *J Immunol* 1982; **128**: 1031-6.
- 20 Rook A H, Tsokos G C, Quinnan G V Jr, *et al.* Cytotoxic antibodies to natural killer cells in systemic lupus erythematosus. *Clin Immunol Immunopathol* 1982; **24**: 179-85.