Serum alpha interferon and lymphocyte inclusions in systemic lupus erythematosus*

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SUMMARY The relationship between serum acid-labile alpha interferon and tubuloreticular inclusions within the cytoplasm of circulating lymphocytes was studied in 46 patients with systemic lupus erythematosus. Elevated levels of interferon (≥ 8 IU/ml) were found in 17 patients and lymphocyte inclusions in 35. The mean serum interferon concentration in patients with lymphocyte inclusions was significantly higher than in patients without inclusions (17.2 versus 2.4 IU/ml, $p < 0.01$). Inclusions were found in 16 of 17 patients with elevated interferon and also in 19 of 29 patients without interferon ($p = 0.026$). In lupus, serum interferon appears to be a sufficient though not an essential marker for the presence of lymphocyte inclusions.

Key words: lupus erythematosus, systemic, interferon type 1, lymphocytes.

The significance of tubuloreticular inclusions (TRI) (Fig. 1) in the cytoplasm of endothelial cells and circulating lymphocytes of patients with systemic lupus erythematosus and various other forms of autoimmunity is unknown.¹ Although the inclusions have been described as virus-like in appearance, the relationship between TRI and viruses, if any exists, has yet to be defined. Cytoplasmic TRI are associated with the endoplasmic reticulum and are ultrastructurally distinct from any known virus.² Cytochemical and autoradiographic studies have shown the inclusions to consist of phospholipid and glycoprotein and not nucleoprotein.³ Moreover, attempts to isolate virus from tissues containing TRI or to correlate their presence with raised levels of serum antibody against several common viruses have been unsuccessful.^{4,5}

Experimentally TRI can be induced in lymphoblastoid cell lines by treatment with the pyrimidine analogue 5-bromo-2'-deoxyuridine.^{6,7} In-vitro stud-

ies of Raji cells treated with halogenated pyrimidines have shown that the synthesis and secretion of a type 1 interferon mediates TRI formation. Furthermore, the direct addition of purified human leukocyte or fibroblast interferon or human serum containing interferon to lymphoblastoid cells resulted in TRI formation.^{8,9} The ability of interferon to induce TRI in vivo has been demonstrated by studies of hepatic endoplasmic reticular aggregates in newborn mice¹⁰ and lymphocyte studies on cancer¹¹ and chronic type B hepatitis¹² patients treated with systemic interferon.

Since both lymphocyte TRI¹³⁻¹⁵ and serum interferon¹⁶⁻²⁰ are present in patients with systemic lupus, we have studied a large number of lupus patients in an attempt to determine the relationship between interferon and lymphocyte inclusions.

Materials and methods

Forty-six patients each of whom satisfied the revised American Rheumatism Association classification criteria for systemic lupus erythematosus²¹ were studied. Lymphocytes were isolated from a heparinised peripheral blood specimen by Hypaque-Ficoll gradient separation. The precise methods for fixation, embedding, cutting, and electron microscopy

*Presented at the VIII Pan American Congress of Rheumatology, Washington, DC, June 1982. Abstract published in *Arthritis Rheum* 1982; 25: S57.

Accepted for publication 7 August 1984.

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Fig. 1 Tubuloreticular inclusion within cytoplasm of circulating lymphocyte of patient with systemic lupus erythematosus. ($\times 49\,500$).

examination of specimens have been previously described.¹³ The samples were designated positive or negative for inclusions after examination of a minimum of 200 separate mononuclear cell cross-sections. No attempt was made to quantitate inclusions in specimens labelled as positive.

Serum interferon was quantitated by semimicro method on human (GM 2504) cell cultures.¹⁸ About 2×10^4 cells were incubated overnight at 37°C with two-fold serial dilutions of serum in 96-well microtitre plates. Encephalomyocarditis virus was added and incubation continued for an additional 24–36 hours. Virus-induced cytopathic effects were evaluated microscopically, and the interferon titre was defined as the reciprocal of the highest dilution of serum to protect 50% of the cells. Standard alpha, beta, and gamma interferons were included in each assay. Results were standardised to NIH No. 023–90–527 reference human leucocyte interferon. A titre of ≥ 8 international units (IU) per millilitre was considered positive. None of 28 normal healthy individuals studied had serum interferon titres which were positive by this convention.

Results

Serum interferon activity ranging from 8 to 100

IU/ml was detected in 17 patients. The interferon activity was shown to be leucocyte, or alpha, interferon by neutralisation with specific antisera and by antiviral activity on bovine (MDBK) cells. Tubuloreticular inclusions were identified in the lymphocytes of 35 patients. The mean serum interferon concentrations were significantly higher in patients with lymphocyte inclusions as compared with patients without inclusions (17.2, SEM 4.4 TRI positive versus 2.4, SEM 0.9 TRI negative, <0.01 , t test) (Fig. 2). Lymphocyte inclusions were found in 16 of the 17 patients with elevated serum interferon (≥ 8 IU/ml). However, inclusions were also found in 19 additional patients in whom serum interferon would generally be considered to be negative (<8 IU/ml). Thus, although a significantly greater proportion of patients with serum interferon had lymphocyte inclusions than those without interferon ($p=0.026$, Fisher's exact test), there was not a perfect correlation between inclusions and serum interferon.

Better to assess the relationship between serum interferon and lymphocyte inclusions sequential studies over periods ranging from 1 to 29 months were done on 13 patients (Table 1). The three patients with the highest levels of interferon (25 to 64 IU/ml) had persistent lymphocyte TRI for

Fig. 2 Interferon concentrations in TRI positive and TRI negative patients. Means $\pm$ standard error are shown with open circles.

follow-up periods up to 17 months. One patient (No. 4) reduced serum interferon from 16 to 4 IU/ml over 11 months yet was found to have lymphocyte TRI on both occasions. Five patients had persistent lymphocyte TRI without detectable levels of serum interferon for up to 26 months of follow-up. Patient 10, followed up monthly for four months, appeared to lose lymphocyte TRI without ever having detectable levels of serum interferon. Finally, three patients with clinically inactive systemic lupus were studied in whom neither interferon nor lymphocyte TRI were ever identified.

Discussion

Tubuloreticular inclusions were first described in the cytoplasm of endothelial cells of patients with

Table 1 Sequential studies of serum interferon and lymphocyte TRI in systemic lupus erythematosus

Patient number	Months from original specimen	Serum interferon (IU/ml)	Lymphocyte TRI + present - absent
A. Interferon positive/TRI positive			
1	—	64	+
	7	25	+
2	—	32	+
	15	25	+
	17	50	+
3	—	50	+
	1	50	+
B. Interferon positive to negative/TRI positive			
4	—	16	+
	11	4	+
C. Interferon negative/TRI positive			
5	—	4	+
	1	4	+
	9	4	+
6	—	4	+
	9	4	+
7	—	4	+
	9	4	+
8	—	4	+
	26	4	+
9	—	4	+
	10	4	+
D. Interferon negative/TRI positive to negative			
10	—	4	+
	2	4	+
	3	4	—
	4	4	—
E. Interferon negative/TRI negative			
11	—	4	—
	29	4	—
12	—	4	—
	13	4	—
	17	4	—
13	—	4	—
	12	4	—
	15	4	—

systemic lupus erythematosus.²²⁻²⁴ Subsequent studies have shown the inclusions not to be specific for lupus but present in various autoimmune,^{25, 26} neurodegenerative,^{27, 28} neoplastic,^{29, 30} and viral³¹ disorders. The cause or biological significance of inclusions in these diseases has never been satisfactorily established.

In lupus inclusions appear within circulating lymphocytes pari passu with clinical and laboratory correlates of disease activity.¹⁴ The stimulus regulating lymphocyte inclusions is unknown. However, from the experimental in-vitro and in-vivo evidence of the induction of inclusions by interferon, interferon must be considered a likely candidate. Multiple types of interferon have been found in the

serum of patients with systemic lupus. The predominant type appears to be a subspecies of alpha interferon which is partially inactivated at pH 2.0.¹⁸ Fluctuating levels of this interferon are reported to correlate with clinical disease activity, antibodies to native DNA, and serum complement.^{16 20}

The present study was an attempt to determine if a relationship exists between serum interferon and lymphocyte inclusions in patients with lupus erythematosus. Elevated serum interferon was found to be a good marker of inclusions in that all but one of 17 patients with elevated interferon were found to have lymphocyte inclusions. However, two-thirds of the patients with inclusions did not have detectable levels of serum interferon. A limited serial study of patients followed up for as long as two years failed to identify clearly a cause and effect relationship between interferon and cytoplasmic abnormalities. Too few patients developing or resolving either interferon or inclusions were identified to bridge states of concordance or discordance. Thus, in lupus erythematosus serum interferon appears to be a sufficient although non essential factor for lymphocyte inclusions.

It is possible that local concentrations of interferon in bone marrow, spleen, lymph nodes, or other organs might be more closely correlated with lymphocyte inclusions. Lymphocyte alterations probably occur in tissues rather than in the circulation. Raised serum levels, although indicative of excessive production, might be too insensitive to detect any cause and effect relationship occurring at the tissue level. Possibly other cellular markers of interferon, such as 2'-5' adenylate synthetase³² might be more closely associated with inclusions.

Recent studies of interferon and lymphocyte inclusions in patients with the acquired immune deficiency syndrome (AIDS) provide interesting contrasts to the studies reported here. Patients with AIDS have an acid labile alpha interferon in their serum which appears to be identical to that found in lupus erythematosus.³³ Tubuloreticular inclusions are present within suppressor/cytotoxic cells reactive with monoclonal anti-Leu 2a antibodies.³⁴ Patients with inclusions have high titres of serum interferon. However, unlike the findings in lupus erythematosus, the increase in serum interferon was not always associated with inclusions. Further studies of these relationships, including the source and biological significance of inducers of lymphocyte inclusions, are likely to be important in our future understanding of autoimmunity.

The authors gratefully acknowledge the contributions of Ms. Emily Klima and Mr Herman Michelitch for technical assistance and Ms. Dawn Smith for typing the manuscript.

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