Variation in circulating immune complex levels with diet, exercise, and sleep: a comparison between normal controls and patients with systemic lupus erythematosus

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SUMMARY Circulating immune complexes (CIC) were measured in 4 normal controls and 8 patients with systemic lupus erythematosus (SLE) by a polyethylene glycol precipitation (PEG) method during a 24-hour period. In all the normal controls levels fell during sleep and after drinking 1 pint (568 ml) of milk and rose before getting out of bed and after moderate exercise. There were also marked differences in the patterns of rise and fall of the CIC levels between the normal controls and patients with SLE. Insufficient account has been taken of these physiological influences in CIC levels in previous studies attempting to relate disease activity to quantitative levels.

Levels of circulating immune complexes (CIC) are frequently used as markers of clinical activity in systemic lupus erythematosus (SLE), although early hopes that they might prove to be useful indicators¹ of inflammatory disease have not been uniformly confirmed.²

There is evidence that CIC levels may be altered by the ingestion of food proteins,³ and it has been noted that bed rest alone seems to reduce the levels of CIC. This investigation is an attempt to explore the possibility that physiological or circadian variations might explain disparities between the levels of CIC and disease activity. The study was performed by measuring CIC levels over a 24-hour period and examining the effects of feeding, sleep and exercise.

Subjects and methods

Four healthy volunteers, 2 male and 2 female, age range 21 to 31 years, mean 28 years, and 4 female patients with SLE were venesected via a 21-gauge butterfly needle during a 24-hour period. The CIC levels were measured at 0730, 1300 and, 1900 h

30 minutes after breakfast, lunch, and supper respectively. Subjects were allowed a free choice of food for lunch and supper but breakfast consisted of 1 pint of milk (568 ml) only. The levels were also measured at 2200 (immediately before going to bed), at 0200 (during sleep), at 0630 (immediately before rising), at 0700 (30 minutes after rising and just before breakfast), at 0900, and at 0915 h (10 minutes after a short period of moderate exercise). One additional patient with SLE went through the above procedure with the exception of the exercise component. The age range of these 5 patients was 25 to 45 years, mean 34 years. Three more female patients with SLE, age range 26 to 35 years, mean 31 years, performed the exercise test only.

Exercise comprised pedalling a bicycle ergometer until the subject's pulse rate was $\frac{2}{3}$ of the maximum for his or her age, as predicted by Åstrand and Rodahl.⁴ This pulse rate was maintained for 3 minutes and the CIC level measured 10 minutes after stopping exercise. Each patient gave her informed consent to the procedures described.

Each of the eight patients satisfied four or more of the American Rheumatism Association criteria⁵ for the classification of SLE. They were each considered to have active disease at the time they were studied.

The CIC levels were measured by a modification⁶ of the polyethylene glycol precipitation technique.⁷

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The IgG content of the redissolved precipitates was assayed by laser nephelometer (Hyland Laboratories, Thetford, Norfolk). Interassay variation is no greater than 3%. The upper limit of normal, established in 30 healthy volunteers, is $100 \mu\text{g ml}^{-1}$ IgG.

Results

The levels of CIC in the 4 volunteers are shown in Fig. 1. Those of the 5 patients with SLE who completed most or all of the procedures are shown in Fig. 2. The CIC levels in the three patients who performed the exercise test only are shown in Table 1.

As might be expected, the CIC levels were invariably higher in the patients with SLE than the normal controls. Only on one occasion did a healthy volunteer have a level exceeding our upper limit of normal. There was, however, considerable variation in the CIC levels in both the normal controls and in

Table 1 Levels of circulating immune complexes before and after exercise (normal range less than $100 \mu\text{g ml}^{-1}$ IgG)

Patients	Before exercise 0900 h	After exercise 0915 h
1	78.5	75.0
2	96.8	86.3
3	288.6	275.4

the patients with SLE during the 24-hour period. The largest variation within a single subject among the normal controls was $69.5 \mu\text{gml}^{-1}$ IgG (average variation during 24 hours of the 4 controls was $54.7 \mu\text{gml}^{-1}$ IgG). Among the lupus patients the figures were $289 \mu\text{gml}^{-1}$ IgG and $141.2 \mu\text{gml}^{-1}$ IgG respectively. Four out of the 5 patients with SLE had at least 1 level of CIC within the normal range recorded during the 24-hour period. The only patient who did not, a 25-year-old woman, had the

Fig. 1 The CIC level in 4 normal subjects during a 24-hour period in response to various physiological stimuli. The upper limit of normal is $100 \mu\text{gml}^{-1}$ IgG.

Systemic Lupus Erythematosus.

Fig. 2 The CIC levels in 5 patients with SLE during a 24-hour period in response to various physiological stimuli. The upper limit of normal is 100 μgml^{-1} IgG.

highest levels of those recorded. During the following 3 months her CIC levels, measured at varying times of day, remained very high but showed considerable fluctuation. Her lowest recorded level in this period was 154 μgml^{-1} IgG and the highest 534 μgml^{-1} IgG.

Table 2 Trends in the levels of circulating immune complexes

	Normal controls	SLE patients
Fall in the CIC levels between 1300 and 1900 h	3/4	2/5
Fall in the CIC levels between 2200 and 0200 h	4/4	5/5
Rise in the CIC levels between 0200 and 0630 h	4/4	2/5
Rise in the CIC levels 30 min after rising	3/4	3/5
Fall in the CIC levels 30 min after drinking 1 pint (568 ml) of milk	4/4	2/5
Rise in the CIC levels 10 min after moderate exercise	4/4	3/7

The patterns of rise and fall in CIC levels showed some distinctive differences between the 2 groups. However, every subject tested showed a fall in CIC levels between 2200 and 0200 h, the average fall for the normal subjects being 22.3 μgml^{-1} IgG and for the lupus group 29.02 μgml^{-1} IgG. The major trends in the normal controls are compared with those of the SLE patients in Table 2.

Discussion

While it is well known that CIC are present in SLE they have also been found in other autoimmune diseases, infectious diseases, parasitic infestations, and also in normal subjects.⁸ It has been suggested that the pathogenicity of CIC may be determined by their size⁹ or the class, subclass, and affinity of the constituent antibody.⁸ Vascular permeability¹⁰ may also be important. Nevertheless a number of attempts

have been made to correlate absolute CIC levels with disease activity in SLE. Using fluid phase C1q binding assays neither Inman *et al.*² nor Abrass *et al.*¹¹ found any correlation of CIC with disease activity. However, Abrass *et al.* did find a statistically significant correlation using a solid phase C1q binding test.

In this study we have shown that physiological mechanisms play a part in determining the level of CIC. The lowering of CIC levels during sleep in all the normal controls and the patients suggests that a circadian rhythm exists. Support for the idea that there is cyclical variation in immunological processes is provided by the work of Cove Smith *et al.*,¹² who showed that the cell mediated response to intradermal tuberculin in normal individuals varied according to the time of day that the challenge was given: the maximal response was at 0700 and the minimal at 2200 h.

Exercise has also been shown to affect the immune response. Short exercise causes a transient lymphocytosis, principally of B cells.¹³ Long-distance running has also been shown to decrease the response of lymphocytes to T and B cell mitogens.¹⁴

In the present study it has been demonstrated that CIC levels alter after feeding, exercise, and change in posture. These findings may have important practical considerations. Immune complex levels in an inpatient fasted overnight and venesected at 0730 h before rising may not reasonably be compared with those of an outpatient who has had breakfast or lunch and who has walked up several flights of stairs before venesection. We thus suggest that samples for CIC should be taken under standard conditions; that without such precautions data may be invalidated; and that the search for other influences that impinge on the levels of CIC be continued.

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