Rosette formation by human T and B lymphocytes in the presence of drugs that raise the cellular levels of cAMP and cGMP. The percentage of inhibition or stimulation was calculated from controls taken as 100%

Drugs	Cases No.	T lymphocytes			B lymphocytes		
		Rosettes %	Inhibition %	Stimulation %	Rosettes %	Inhibition %	Stimulation %
None	65	53.3± 7.0	–	–	20.8±6.7	–	–
Theophylline $10^{-3}M$	29	41.0± 9.5[a]	20	–	25.0±8.6[b]	–	28
Papaverine $10^{-3}M$	23	17.0±11.3[a]	68	–	26.0±8.1[a]	–	36
Isoproterenol $2{\cdot}10^{-4}M$	13	48.3± 9.7[a]	21	–	31.3±7.3[a]	–	34
Dibutyryl cAMP $2{\cdot}10^{-4}M$	13	48.6± 9.0[a]	24	–	29.4±5.8[a]	–	27
Isoproterenol $2{\cdot}10^{-4}M$ + propranolol $10^{-3}M$	11	52.0± 8.0	NS	–	21.2±5.0	–	NS
None	24	47.8± 6.9	–	–	23.3±6.5	–	–
Pilocarpine $10^{-3}M$	9	55.4± 6.4[a]	–	12	16.0±2.7[a]	33	–
Carbamylcholine 10^{-6}	10	55.0± 6.7[a]	–	14	17.0±2.6[c]	27	–
Dibutyrol cGMP $2{\cdot}10^{-4}M$	10	53.0± 4.1[b]	–	11	15.5±3.5[a]	32	–
Carbamylcholine $10^{-6}M$ + atropine $10^{-6}M$	8	47.0± 6.8	–	NS	22.0±3.7	NS	–

[a] $p < 0.005$; [b] $p < 0.025$; [c] $p < 0.01$; NS = not significant.

stroyed by trypsin but is not blocked by antibody to Ig. Human thymocytes and T lymphocytes, on the other hand can be identified by surface membrane receptors which bind to SRBC to form non-immunological E-rosettes. They are dependent on the concentration of divalent cations[8], can be enhanced by treatment of lymphocytes by neuraminidase[9], and are inhibited by iodacetate[8], trypsin[8], azide[10], antilymphocyte serum[10], cytochalasin B[11]. The properties of human thymocytes and T lymphocytes of making spontaneous rosettes with SRBC appears to be conferred on T-cell precursors[12,13]. Curiously, it was found that theophylline and thymus extracts, known to increase the intracellular levels of cAMP, both have the capacity of stimulating E-rosette formation by human precursors lymphocytes and of restoring the immunological competence of neonatally thymectomized mouse. The results obtained in our experiments with rosette formation, suggest that cyclic nucleotides are modulator agents of lymphocyte membrane function. These nucleotides might be exerting their effects by modulating the synthesis of membrane receptors for SRBC and C3, by incorporating these receptors into plasma membranes or even by shedding this receptors from the lymphocyte membranes.

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Serum Immunoconglutinins and Complement in Systemic *Lupus erythematosus*[1]

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Summary. IK activity titrated by the sedimentation method in sera from patients affected with SLE was found to be negatively correlated with C4 and C3 complement factors levels. The significance of this data is discussed.

Generally the immunoconglutinins, (considered as autoantibodies with anti-complement specificity) formation reflects an in vivo complement fixation[2]. The IKs react specifically with fixed C3 and C4, in the KAF-unreacted form, i. e., C3b and C4b; once bound, IKs can trigger a new complement activation and so fix more C3 and C4[2]. These auto-antibodies are predominantly IgM, and the presence of free complement components, as well as EDTA, did not inhibit their reaction. On the contrary IK activity found in human saliva is referred to an unusual IgA class of antibody[3], and it is inhibited by free complement components or EDTA in solution. Thus, these two latter properties make the salivary IKs functionally similar to bovine conglutinins (non antibodies), which reacts in an EDTA reversible way, with the KAF-reacted form of C3(C3d)[2].

Serum raised IK values have been reported in many human pathological states: overall in infections[4,5], and in collagen-vascular diseases[6–8]; in all these conditions, a certain degree of complement consumption is usually found. Up to the present, the exact relation between IKs rizing and complement lowering, from the clinical standpoint, has not been investigated except for some cases of renal pathology[9–11].

We valued the IK titre and some complement functions in patients affected with Systemic *Lupus erythematosus*,

Table I. IK and complement factors values in SLE and control (mean value ± SE)

	IK titre log	CH50 HU/ml	C4 mg%	C3 mg%	C3PA mg%
Control	1.92 ± 0.31	938 ± 18.15	28.27 ± 1.36	103.4 ± 3.24	16.8 ± 0.63
SLE	6.25 ± 0.47	550 ± 76.15	17.66 ± 1.98	95.75 ± 7.74	22.5 ± 1.6
p	<0.01	<0.01	<0.01	–	–

Table II. IK/complement and CH50/complement factors correlation in SLE

	r_s	p		r_s	p
IK/C4	−0.376	0.05	CH50/C4	+0.787	<0.01
IK/C3	−0.316	0.05	CH50/C3	+0.554	<0.01
IK/CH50	−0.174	–	CH50/C3PA	+0.19	–
IK/C3PA	+0.266	–			

in order to verify the quantitative reports between complement consumption and IKs formation.

Materials and methods. 28 serum specimens were collected from 10 patients affected with SLE: 4 patients had more than 3 consecutive specimens; a control group was constituted of 20 healthy donors. In each specimen IK, CH50, C4, C3, C3PA evaluations were performed. IK activity was titrated by the sedimentation method by using EAC43 Antrypol (sheep erytrocytes sensitized by rabbit IgM antibodies, and alexinated so that the C3 molecules were predominantly in C3b form) as indicator system, according to LACHMANN et al.[12]. Total haemolitic activity (CH50) was evaluated by the modifications of MAYER's method[12]. C4, C3, C3PA concentrations were evaluated by single radial immunodiffusion[13] by rabbit antisera (Behringwerke). Statistical analysis was performed by Student's t-test and r_s Spearman's correlation coefficient.

Results and discussion. The IK titre, CH50 and C4 values were significantly different ($p < 0.01$) in the 2 groups studied, but this was not the case with C3 and C3PA (Table I). This is a consequent finding in SLE, if one considers that in such a disease the complement is consumed by the 'classical' pathway. Although C3 level was not significantly lowered, it must be emphasized that its value, when related to CH50 ($p<0.01$) and C4 ($p<0.01$), showed a relative lowering (Table II). The Spearman's coefficient indicated a significant correlation between IK rising and C4 ($p<0.05$) or C3 ($p\sim0.05$) fall; on the other hand, IK titre did not show any accord with CH50 or C3PA value (Table II).

Our data suggest a significant correlation between C4 and C3 fall at the same time as IK rizing activity in the case of a 'classic' complement activation. In effect C3 and C4 are the 'IK-gens' of the IK activity. If we consider that IK has the kinetics of an immunological response, it is difficult to refer to the relation between the complement fall the IKs increased formation, at the level of each single specimen.

Our data should be explicable by the condition of a chronic complement consumption, as it happens in SLE. From this standpoint, we are forced to consider the rizing IK titre as a sensitive index, at least as C4 and C3 fall, indicating a previously occurring complement fixation.

Finally IKs, as a clinical warning factor, could be important if complement consumption has been poor or erratic over a long period of time, or if the 'classic' and 'alternate' pathways are contemporaneously and only slightly triggered, so that they are not able to produce a significant C4, C3PA and C3 consumption.

[1] Index of the abbreviations used: SLE, systemic *Lupus eritematosus;* IK, immunoconglutinins; CH50, Total haemolytic activity; C4b, Complement factor connect with the complex AgAbCl; C3b, Complement factor connect with the complex AgAbClC4C2; C3PA, C3 proactivator in the complement activation by the alternate pathway implicated; KAF, Conglutinogen activating factor inactive the C3b in C3c and C3d. Antrypol, factor that makes C3b KAF-unreactive.

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