MODULATION OF HUMAN LYMPHOCYTE FUNCTION
BY C3a AND C3a(70–77)*

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The possibility that the third component of complement, C3, might regulate immune responses and other specific activities of lymphocytes was suggested initially by the effects on lymphocyte function of complement depletion in vivo (1) and the introduction of C3 in vitro (2–5). C3 and partially purified C3a, the fragment of C3 that exhibits anaphylatoxin activity (6), inhibit the transformation of rat and human lymphocytes incubated with mitogenic lectins in serum-free medium, as assessed by suppression of the increased lymphocyte uptake of [^{3}H]thymidine (5, 7). Analyses of the cellular specificity of partially purified C3a have indicated that the proliferative responses of rat B lymphocytes are more susceptible to inhibition than those of T lymphocytes (7). Both purified human C3a and the synthetic octapeptide C3a(70–77), which retains the activities of an anaphylatoxin (6, 8), now are shown to interact directly with human lymphocytes, as evidenced both by selective inhibition of the T lymphocyte migration and lymphokine generation elicited by diverse stimuli and by the reduced lymphokine-generating activity of lymphocytes exposed to solid-phase C3a(70–77).

Materials and Methods

Medium 199, Hanks' balanced salt solution, Eagle's minimal essential medium, a MASH II cell harvester (M. A. Bioproducts, Walkersville, MD), activated CH-Sepharose 4B, Sephadex G-10, 25, 75, and 100, SP-Sephadex, QAE-Sephadex, macromolecular dextran (Macrodex), and Ficoll-Hypaque (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, NJ), 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (Hepes), penicillin G, streptomycin (Gibco Laboratories, Grand Island Biological Co., Grand Island, NY), five times recrystallized ovalbumin, concanavalin A (Con A)[1] (Miles Laboratories, Inc., Elkhart, IN), phytohemagglutinin-M (PHA) (Difco Laboratories, Inc., Detroit, MI), streptokinase-streptodornase (SK-SD) (Lederle Laboratories Inc., Pearl River, NY), α-thioglycerol (Sigma Chemical Co., St. Louis, MO), Drummond glass capillary tubes (Bolab, Inc., Derry, NH), murine monoclonal antibodies to human leukocyte surface antigens (Ortho Diagnostics, Inc., Westwood, MA) (9), MacKaness-type plastic chambers (Adaps, Inc., Dedham, MA), [^{3}H]thymidine (New England Nuclear, Boston, MA), 3-μm pore and 8-μm pore membrane filters (Sartorius Filters, Hayward, CA), 8-μm channel filters (Nuclepore Corp., Pleasanton, CA), and chemotactic chambers with a 0.2-ml blind-end stimulus compartment (Neuroprobe, Inc., Bethesda, MD) were obtained from the

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[1] *Abbreviations used in this paper:* Con A, concanavalin A; HBSS-OA, Hanks' balanced salt solution containing 0.2 g ovalbumin/100 ml; LIF, leukocyte inhibitory factor; PHA, phytohemagglutinin; PMN, polymorphonuclear leukocytes; SK-SD, streptokinase-streptodornase.

specified suppliers.

Chemotactic fragments of the fifth component of human complement (C5fr) were purified from zymosan-activated human serum as described (10). C3a was generated by incubating fresh human serum with 10 mg/ml of zymosan in the presence of 1 M ϵ-amino-caproic acid and purified by sequential filtration on Sephadex G-100 in 0.1 M ammonium formate (pH 5.0), ion-exchange chromatography on SP-Sephadex and QAE-Sephadex, and gel filtration on Sephadex G-75 in 0.05 M ammonium formate (pH 5.0) (11). The C3a was quantified by a standard protein assay (Bio-Rad Laboratories, Richmond, CA), its capacity to contract segments of guinea pig ileum, and reactivity with monospecific anti-human C3 in a standard immunodiffusion assay (6, 11). The octapeptide Ala-Ser-His-Leu-Gly-Leu-Ala-Arg, C3a(70–77), was prepared by standard solid-phase synthesis techniques and purified by filtration on Sephadex G-25 in 0.01 M acetic acid and counter-current distribution (12). C3a(70–77) was coupled by the terminal amino-group to CH-Sepharose 4B by reacting 1 g of activated CH-Sepharose 4B with 10 μmoles of C3a(70–77) in 5 ml of 0.5 M NaCl-0.1 M NaHCO$_3$ (pH 7.4) for 1 h at room temperature under a N$_2$ atmosphere. The product was washed twice with 200 ml each of 1 M NaCl-0.1 M sodium acetate (pH 4.0), 1 M NaCl-0.1 M Tris-HCl (pH 8.0), and distilled water and stored in 0.01 M acetic acid at 4°C under N$_2$ for up to 1 wk. Amino acid analyses of portions of the gel that had been hydrolyzed for 8 h at 160°C in propionic acid:HCl (1:1; vol/vol) indicated a coupling of 2.6–2.8 μmoles of C3a(70–77) per ml of CH-Sepharose 4B (range, $n = 3$). CH-Sepharose to which had been coupled the same quantity of glycine served as the adsorbent in control columns.

Isolation of Human Leukocytes. Leukocytes from normal donors were obtained and processed under sterile conditions for the production and quantification of leukocyte inhibitory factor (LIF) activity in the glass capillary tube assay and the assessment of lymphocyte proliferation. After dextran sedimentation of the erythrocytes in heparinized blood, the mixed leukocytes in the plasma were sedimented by centrifugation, washed, and resuspended in medium 199 containing 25 mM Hepes (pH 7.4), penicillin (100 U/ml), and streptomycin (100 μg/ml) (M199-HPS) (13). 10-ml portions of 2 × 10^6 leukocytes/ml were layered on 10-ml Ficoll-Hypaque cushions (sp gr, 1.076) and centrifuged at 500 g for 35 min at room temperature (13). The mononuclear leukocytes at the buffer-gradient interface were harvested and washed twice in M199-HPS. The polymorphonuclear leukocytes (PMN) in the pellet were resuspended in 1 ml of AB-positive human serum that was diluted to 10 ml with distilled water at room temperature to lyse contaminating erythrocytes. After 20 s, 40 ml of M199-HPS was added, and the PMN were recovered by centrifugation and washed twice with M199-HPS. The purity of the preparations was >95% for the PMN and >93% for the mononuclear leukocytes.

PMN and T lymphocytes for studies of leukocyte migration in the modified Boyden chamber assay were isolated from citrate-anticoagulated venous blood of normal subjects, as described (10, 14). The mixed leukocytes were incubated in 0.84 g/100 ml NH$_4$Cl with 0.01 M NaHCO$_3$ (pH 7.2) for 2 min at room temperature to lyse contaminating erythrocytes, washed, resuspended in Hanks' solution containing 0.2 g ovalbumin/100 ml (HBSS-OA) and centrifuged on cushions of Ficoll-Hypaque to yield PMN of >95% purity and mononuclear leukocytes (15). The PMN were washed and resuspended in HBSS-OA. 5-ml portions of the suspension of 4 × 10^7 mononuclear leukocytes/ml in Eagle's medium with 0.2 g ovalbumin/100 ml were each applied to a column of 10 ml of Sephadex G-10 on 5 ml of nylon wool that was equilibrated and developed with 45 ml of the same medium. The leukocytes in the effluent were 92–96% T lymphocytes (range, $n = 6$), as assessed by quantifying the rosetting of sheep erythrocytes without and with surface C3b or C3d and by analyzing the surface immunoglobulins (14). For the preparation of T lymphocytes for LIF studies, mononuclear leukocytes from Ficoll-Hypaque cushions were incubated with neuraminidase-treated fresh sheep erythrocytes to achieve rosetting. The T lymphocytes were recovered from Ficoll-Hypaque cushions after lysis of the erythrocytes by hypotonic exposure, as described (16); the purity of the T lymphocytes always exceeded 95%.

Purification and Quantification of LIF. Replicate 2-ml suspensions of 6–8 × 10^6 mononuclear leukocytes or purified T lymphocytes per ml in M199-HPS containing 10% (vol/vol) AB-positive serum were cultured without or with 10 μg/ml of Con A, PHA, or SK-SD for 24 h at 37°C in a humidified atmosphere of 5% CO$_2$:95% air. Unless otherwise indicated, C3a or

C3a(70–77) was added to cultures at the same time as the mitogen or antigen stimulus. The same concentration of stimulus was added to control cultures at 24 h, immediately before centrifugation of all of the cultures at 1,050 g for 10 min at room temperature to obtain leukocyte-free supernatants.

Sterile suspensions of PMN were washed and resuspended in 6 volumes of M199-HPS containing 15% (vol/vol) AB-positive human serum. 20-μl sterile glass capillary tubes were filled with the PMN suspension, centrifuged, cut at the level of the PMN pellet, and placed in duplicate on glass cover slips in MacKaness-type plastic chambers that were filled with LIF-containing or control supernatant and incubated for 8 h at 37°C (13, 17). The area of migration of PMN from each capillary tube was quantified by projection microscopy and planimetry, as described (13), and the LIF activity in stimulated supernatants was calculated by the formula: mean percent inhibition of migration = 1 − ([mean area of migration from four capillaries in supernate of stimulated cultures]/[mean area of migration from four capillaries in supernate of control cultures]) × 100.

PMN chemotaxis was assessed in modified Boyden chambers with C5fr as the stimulus, and the responses were quantified by microscopic counting of the PMN in fixed and stained filters, as described (10, 17). Chemotactic responses were expressed as mean net PMN per high-power field after subtraction of the mean value for filters of chambers lacking C5fr. To measure the activity of LIF, PMN were incubated for 10 min at 37°C with 1/10 dilutions of supernatant from stimulated and control cultures before adding the leukocytes to the chemotactic chambers. Mean percent inhibition of migration in duplicate chambers was calculated as for the capillary tube method. The mean percent inhibition of migration by supernatants with C3a or C3a(70–77) was corrected for the level of inhibition observed when the same concentration of peptide alone was added to the assay, which never exceeded 20% in the capillary tube method or 5% in the chemotactic method.

Assessment of Lymphocyte Proliferation. 2 × 10⁵ mononuclear leukocytes in 0.2 ml of M199-HPS containing 15% (vol/vol) AB-positive serum were added to each well of microtiter plates without or with 10 μg/ml of Con A, PHA, or SK-SD and incubated at 37°C in 5% CO_2:95% air. C3a or C3a(70–77) was introduced into some suspensions at the same time as the stimulus or buffer. After 3 d for the mitogen-stimulated cultures and 5 d for the SK-SD-stimulated cultures, 1 μCi of [³H]thymidine was added to each well. After an additional 16 h at 37°C, the uptake of [³H]thymidine was analyzed by aspirating the contents of each well with a Mash II harvester that collected and washed the leukocytes on glass fiber filters, as described (18). The radioactivity in each filter was quantified, and the results for quadruplicate chambers were expressed as mean cpm ± SEM. The percentage inhibition of lymphocyte uptake of [³H]thymidine by C3a or C3a(70–77) was calculated from the ratio of the net uptake of radioactivity of stimulated lymphocytes in the presence of peptide to that in the absence of peptide (5).

Measurement of T Lymphocyte Migration. T lymphocyte migration was quantified in modified Boyden chambers. The upper compartment of each chamber, containing 0.5 ml of a suspension of 5–6 × 10⁶ T lymphocytes in HBSS-OA, was separated from the lower compartment containing 0.2 ml of HBSS-OA by a layer consisting of an 8-μm pore Nuclepore filter over an 8-μm pore Sartorius filter (14). C3a or C3a(70–77) without or with Con A or α-thioglycerol was added at equal concentrations to both compartments, and the chambers were incubated for 4 h at 37°C in 5% CO_2:95% air. The T lymphocytes that migrated in the front at 60–80 μm from the cell source were enumerated in 10 high power fields in filters from duplicate chambers. The migration of T lymphocytes in the presence of C3a or C3a(70–77) is expressed as a percent of that in buffer or a stimulus alone. The levels of significance of the differences in all assays were analyzed by the standard Student's t test.

Results

Inhibition of the Generation of LIF Activity by C3a and C3a(70–77). Incubation of normal human mixed mononuclear leukocytes with the mitogens PHA and Con A or the antigen SK-SD for 24 h at 37°C resulted in the generation of substantial levels of LIF activity, as assessed by the capacity of the culture supernatants to inhibit the

migration of human PMN from glass capillary tubes (Figs. 1-3). The introduction of C3a or C3a(70-77) into the mononuclear leukocyte cultures along with the mitogen or antigen at the onset of incubation resulted in a peptide concentration-dependent inhibition of the generation of LIF activity, irrespective of the stimulus. The extent of suppression of the generation of LIF activity was significant at concentrations of C3a(70-77) of 10^{-7} M or greater for each of the stimuli and exceeded 75% suppression at 10^{-6} M C3a(70-77) (Figs. 1-3). The concentration of C3a(70-77) required to achieve 50% inhibition of LIF generation (IC_{50}) was $\sim 10^{-8}$ M for Con A and PHA and 10^{-7} M for SK-SD. The suppression by C3a of the generation of LIF activity by mononuclear leukocytes exhibited a similar peptide concentration-suppression relationship as that observed with C3a(70-77) for the mitogen stimuli (Figs. 1 and 2), as assessed by the examination of IC_{50} values. The potency of C3a was more than
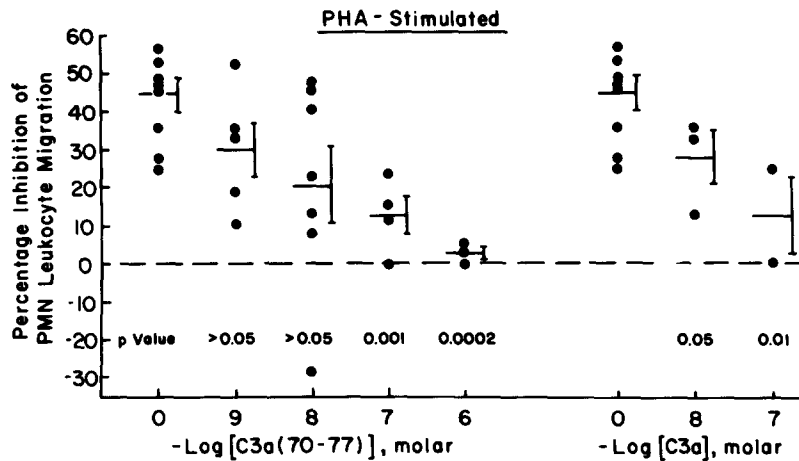

Fig. 1.   Effect of C3a and C3a(70-77) on the generation of LIF activity by human peripheral blood mononuclear leukocytes stimulated with PHA. LIF was quantified by the inhibition of human PMN migration from glass capillary tubes. Each bar and error bracket represents the mean ± SEM for the group of data points. Each P value indicates the level of significance of the difference between the samples with PHA and C3a or C3a(70-77) and those with PHA alone.
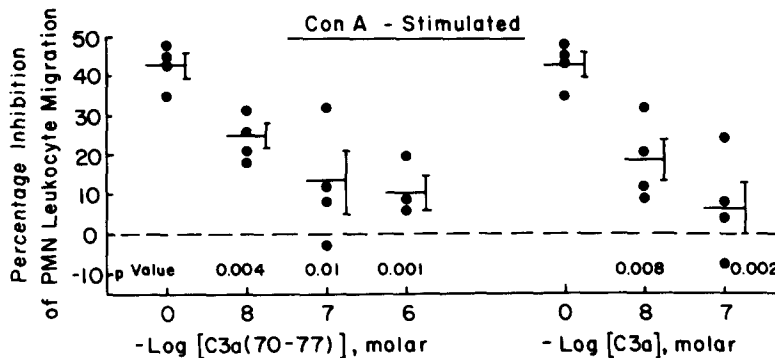

Fig. 2.   Effect of C3a and C3a(70-77) on the generation of LIF activity by human peripheral blood mononuclear leukocytes stimulated with Con A. LIF was quantified by the inhibition of human PMN migration from glass capillary tubes. Each bar and error bracket represents the mean ± SEM for the group of data points. Each P value indicates the level of significance of the difference between the samples with Con A and C3a or C3a(70-77) and those with Con A alone.
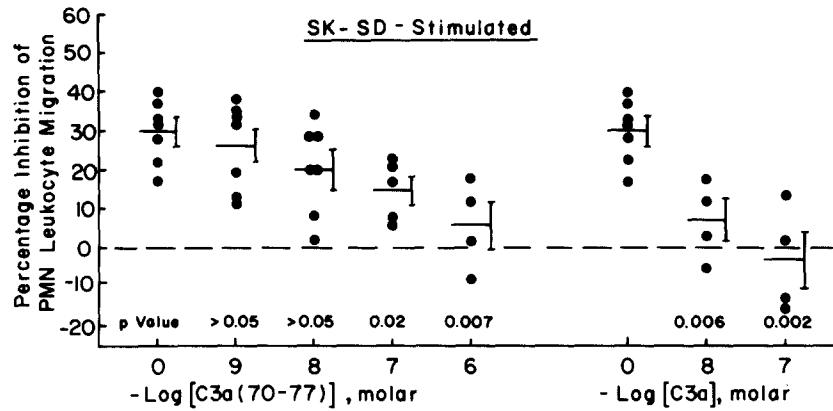
FIG. 3.   Effect of C3a and C3a(70–77) on the generation of LIF activity by human peripheral blood mononuclear leukocytes stimulated with SK-SD. LIF was quantified by the inhibition of human PMN migration from glass capillary tubes. Each bar and error bracket represents the mean ± SEM for the group of data points. Each P value indicates the level of significance of the difference between the samples with SK-SD and C3a or C3a(70–77) and those with SK-SD alone.
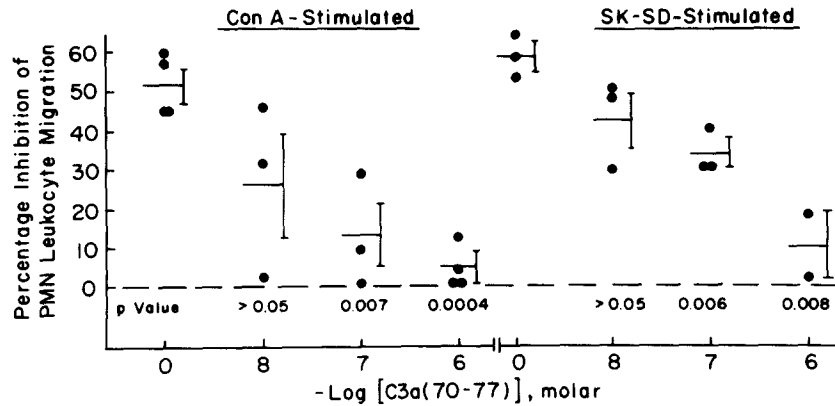

FIG. 4.   Effect of C3a(70–77) on the generation of LIF activity by human peripheral blood mononuclear leukocytes as measured by the inhibition of human PMN chemotaxis to C5fr in Boyden chambers. Each bar and bracket represents the mean ± SEM for the group of data points. Each P value indicates the level of significance for the same comparisons as in Figs. 2 and 3.

10-fold higher than that of C3a(70–77) with respect to the suppression of LIF generation evoked by the antigen SK-SD (Fig. 3). Quantification of LIF activity by the inhibition of chemotaxis of PMN to C5fr in a Boyden chamber assay corroborated the results of the capillary tube assay for LIF, despite the greater inhibitory activity of LIF in the former assay (Fig. 4). The IC_{50} for the suppression of LIF generation was $\sim 10^{-8}$ M C3a(70–77) with Con A as the stimulus and was 10^{-7} M C3a(70–77) with SK-SD, which were identical to the respective values obtained with the capillary tube assay.

When C3a(70–77) was added to the cultures either 1 h before or after PHA or Con A, the extent of suppression of the generation of LIF activity elicited by either mitogen was not significantly different from the results in the standard experimental protocol, where the mitogen and C3a(70–77) were added together at the beginning of the 24-h incubation interval. That the suppressive effect of C3a and C3a(70–77) was attrib-

utable to inhibition of the generation or release of LIF, rather than interference with the expression of LIF activity, was demonstrated by two separate approaches. First, the addition of 10^{-9} M to 10^{-6} M C3a(70–77) or of 10^{-8} M to 10^{-6} M C3a to LIF-containing supernatants after removal of the mononuclear leukocytes did not diminish the LIF activity detected in either the capillary tube or Boyden chamber assay. Second, the amount of LIF recovered by gel filtration from supernatants of cultures stimulated by mitogen without or with C3a(70–77) was directly proportional to the quantity found by assays of the unpurified supernatants before the resolution of LIF from C3a(70–77). Two sets of duplicate supernatants were prepared from 2×10^8 mononuclear leukocytes in 60 ml of M199-HPS containing 10% AB-positive serum and 10 μg/ml of PHA, one set of which contained 10^{-6} M C3a(70–77). The supernatants were lyophilized, redissolved in 3 ml of distilled water, dialyzed against distilled water for 24 h at 4°C, and filtered on a column of Sephadex G-100 that was equilibrated and developed with M199-HPS as described (17). The portions of the effluent that contained LIF activity were pooled and concentrated by pressure filtration (Amicon, Inc., Bedford, MA) to 3 ml. The LIF activity detected by the capillary tube method was 43 ± 3% (mean ± SEM) for the set without C3a(70–77) and 16 ± 2% for the set with 10^{-6} M C3a(70–77) before the removal of C3a(70–77) by gel filtration. The respective values were 40 ± 2% and 20 ± 2% for a $\frac{1}{100}$ dilution of the pools of LIF after gel filtration.

In two experiments with T lymphocytes purified by the erythrocyte rosetting technique, the LIF activity elicited by 10 μg/ml of PHA induced 20% and 20% inhibition of PMN migration from glass capillary tubes, which was suppressed to 1% and −10%, respectively, by 10^{-8} M C3a(70–77).

Inhibition of T Lymphocyte Migration by C3a(70–77). The addition of 10^{-9} M to 10^{-6} M C3a(70–77) to purified human T lymphocytes suppressed the enhanced random migration elicited by 1 μg/ml of Con A and 3×10^{-6} M α-thioglycerol in a concentration-dependent manner (Table I). The extent of inhibition of T lymphocyte migration was significant at or above concentrations of C3a(70–77) of 3×10^{-9} M for the Con A stimulus and of 10^{-9} M for α-thioglycerol. The IC$_{50}$ for C3a(70–77) was $\sim 3 \times 10^{-8}$ M with Con A and 10^{-8} M with α-thioglycerol.

Lack of Effect of C3a and C3a(70–77) on Lymphocyte Proliferation and Viability. The addition of 10^{-8} M to 10^{-6} M C3a(70–77) or 10^{-8} M and 10^{-7} M C3a to suspensions of mononuclear leukocytes without or with PHA, Con A, or SK-SD at the beginning

TABLE I

Inhibition of Human T Lymphocyte Migration by C3a(70–77)

Stimulus of migration	Concentration of C3a(70–77), molar							
	3×10^{-10}	10^{-9}	3×10^{-9}	10^{-8}	3×10^{-8}	10^{-7}	3×10^{-7}	10^{-6}
	Percentage of control-stimulated migration							
	mean ± SD; n = 3							
Con A	—	90 ± 8	76 ± 11*	57 ± 12*	49 ± 10*	44 ± 11*	—	—
α-thioglycerol	96 ± 3	83 ± 2*	72 ± 4*	53 ± 5*	43 ± 5*	39 ± 8*	33 ± 6*	26 ± 4*

The level of statistical significance of the inhibition of migration by C3a(70–77) relative to the corresponding control stimulated migration of T lymphocytes in the absence of C3a(70–77) (100%) is denoted by an asterisk; $P < 0.01$.

of the incubation period failed to suppress significantly the level of uptake of [^{3}H]thymidine (Table II). The incubation of mononuclear leukocytes with 10^{-6} M C3a or 10^{-6} M C3a(70-77) for 2-12 h failed to reduce the viability of the leukocytes below that of those in medium alone, as assessed by the exclusion of trypan blue dye and the level of uptake of [^{3}H]thymidine in culture.

Adsorption of LIF-producing Mononuclear Leukocytes on C3a(70-77)-Sepharose. To examine the interaction of mononuclear leukocytes with C3a(70-77) more directly, identical aliquots of suspensions of mononuclear leukocytes were filtered over a column of Sepharose to which had been coupled C3a(70-77) and a control column of Sepharose bearing the same molar quantity of glycine. The mean levels of LIF activity generated by Con A stimulation of leukocytes in the effluent from control Sepharose columns were 42%, 26%, and 32% inhibition of PMN migration, respectively, in three separate experiments, whereas the LIF activity generated by Con A stimulation of the mononuclear leukocytes in the effluents from C3a(70-77)-Sepharose columns were 0%, −10%, and −14%. In each of the three experiments, one-half of the leukocytes in the effluents from the C3a(70-77)-Sepharose column and from the control column were mixed together before stimulation with Con A. The mean levels of LIF generation were 36%, 35%, and 39% inhibition of PMN migration, which were not significantly different from the levels observed with the same number of mononuclear leukocytes from control columns. In addition, 10^{-6} M C3a(70-77) suppressed the levels of generation of LIF activity to 9 ± 9% (mean ± SEM; $n = 3$; $P < 0.07$) for leukocytes from control columns and to 4 ± 7% ($P < 0.01$) for the mixtures of leukocytes from control and C3a(70-77)-Sepharose columns. The viability of the mononuclear leukocytes in the effluents ranged from 80-85% for all column procedures, as assessed by the exclusion of trypan blue dye, and the recovery ranged from 85-90%.

The distribution of subclasses of T lymphocytes in the column effluents for two consecutive experiments was analyzed in a fluorescence-activated cell sorter (Ortho Spectrum III; Ortho Diagnostic Systems, Westwood, MA) with standardized mouse monoclonal antisera to human leukocyte antigens (9). The subpopulations of T lymphocytes in the effluents from the control-Sepharose column were 41.9% and 52.2% OKT4, 67.7% and 82.1% OKT3, and 34.2% and 24.3% OKT8, and in the

TABLE II

Effect of C3a and C3a (70-77) on the Proliferative Responses of Human Blood Mononuclear Leukocytes

C3a(70-77)	C3a	Stimulus	PHA (6)*		Con A (8)		SK-SD (7)	
			mean cpm ± SEM	% inhibition‡	mean cpm ± SEM	% inhibition‡	mean cpm ± SEM	% inhibition‡
—	—	—	400 ± 36		580 ± 118		2,843 ± 897	
10^{-8} M	—	—	372 ± 41		674 ± 129		2,640 ± 1,000	
10^{-7} M	—	—	452 ± 62		609 ± 104		2,610 ± 1,048	
10^{-6} M	—	—	369 ± 32		580 ± 104		2,886 ± 968	
—	10^{-8} M	—	712 ± 186		930 ± 251		4,246 ± 1,413	
—	10^{-7} M		618 ± 92		1,258 ± 327		3,450 ± 1,052	
—	—	+	214,195 ± 5,093		107,417 ± 5,868		41,274 ± 15,851	
10^{-8} M	—	+	205,446 ± 8,762	5 ± 3	88,289 ± 4,916	7 ± 2	35,532 ± 13,892	24 ± 9
10^{-7} M	—	+	224,289 ± 7,831	0	101,477 ± 4,190	6 ± 2	37,798 ± 14,992	11 ± 5
10^{-6} M	—	+	212,635 ± 11,279	4 ± 4	102,652 ± 5,870	6 ± 3	39,734 ± 18,569	21 ± 8
—	10^{-8} M	+	214,160 ± 10,213	5 ± 3	97,776 ± 5,431	10 ± 4	33,069 ± 14,150	32 ± 12
—	10^{-7} M	+	201,225 ± 19,917	10 ± 8	96,681 ± 6,526	12 ± 6	32,675 ± 15,328	39 ± 15

* The number of experiments performed is indicated in parentheses.
‡ In no instance was the percent inhibition statistically significant.

respective effluents from the C3a(70–77)-Sepharose column were 36.6% and 44.1% OKT4, 69.5% and 80.6% OKT3, and 33.8% and 22.7% OKT8. The subpopulations of T lymphocytes in the effluents from control columns were not different from those found in the circulation (9). Thus, the interaction of mononuclear leukocytes with solid-phase C3a(70–77) resulted in a relative depletion of the helper/inducer subpopulation (OKT4), without significant alterations in the suppression/cytotoxic subpopulation (OKT8) or in the overall representation of lymphocytes (OKT3).

Discussion

Diverse activities of lymphocytes are regulated with a high degree of specificity by numerous immunological mediators other than antigens and antibodies. Definitive characterization of the mechanisms of such lymphocyte responses requires the use of structurally defined principles. The direct interaction of purified C3a and C3a(70–77) with human mononuclear leukocytes in culture resulted in a concentration-dependent inhibition of the generation of LIF evoked by mitogens and by the antigen SK-SD (Figs. 1–4). The extent of suppression of LIF generation by C3a(70–77) was significant at concentrations of 10^{-7} M or higher and exceeded 75% at 10^{-6} M, irrespective of the stimulus. The same C3a(70–77) concentration-inhibition relationships were obtained whether LIF was quantified with the capillary tube assay or a modified Boyden chamber method. That C3a(70–77) was capable of suppressing distinct immunological activities of isolated human T lymphocytes was demonstrated by two separate approaches, although an action on mononuclear phagocytes has not been excluded. First, the generation of LIF by T lymphocytes purified with an erythrocyte rosetting technique was inhibited by 10^{-8} M C3a(70–77) to a similar degree as the generation of LIF by mixed mononuclear leukocytes. Second, the migration of T lymphocytes stimulated by Con A or by α-thioglycerol was suppressed significantly by C3a(70–77). The IC_{50} of 3×10^{-8} M C3a(70–77) with Con A as the stimulus for migration (Table I) was similar to the IC_{50} of 10^{-8} M C3a(70–77) observed for the generation of LIF elicited by Con A (Fig. 2).

The inhibitory effects of C3a on lymphocytes exhibited both functional and molecular specificity. At concentrations that suppressed maximally the generation of LIF, neither C3a nor C3a(70–77) inhibited significantly the proliferative responses of mononuclear leukocytes to mitogens or antigen (Table II). Although C3a(70–77) retains only 1–2% of the potency of intact C3a with respect to anaphylatoxin activity (6, 9), the generation of LIF by mononuclear leukocytes and T lymphocytes cultured with mitogens was suppressed similarly by synthetic C3a(70–77) and purified C3a (Figs. 1, 2). In contrast, the potency of C3a was more than 10-fold higher than that of C3a(70–77) in terms of the suppression of LIF generation by mononuclear leukocytes (Fig. 3) and T lymphocytes cultured with the antigen SK-SD. If it is assumed that the capacity of C3a to distinguish between antigen- and mitogen-stimulated lymphocytes is not a function of other factors contaminating the C3a preparation, then it may be concluded that the regulatory effects of C3a reside in more than one molecular domain. Only amino acids 70–77 are required for optimum modulation of lymphokine production by mitogen-stimulated lymphocytes, whereas one or more other domains of C3a might need to be presented to antigen-stimulated lymphocytes for maximum inhibitory potency.

The ability of a C3a(70–77)-Sepharose column to adsorb mononuclear leukocytes

critical to the generation of lymphokines was demonstrated by the failure of the lymphocytes in the column effluent to generate LIF when challenged with Con A. That the loss of LIF-generating activity was not a function of the production of an inhibitory factor was proven by the findings of similar levels of LIF in supernatants from control lymphocytes and from mixtures composed of an equal number of control lymphocytes and of lymphocytes passed through a C3a(70–77)-Sepharose column. The data presented do not definitively exclude the possibility that the LIF-generating lymphocytes were inactivated, but not adsorbed, on the C3a(70–77)-Sepharose column.

The secondary antibody responses of rat lymph node cells to both thymus-dependent and thymus-independent antigens in vitro were inhibited by purified human C3 and fragments of C3 that resembled C3a in molecular weight (7). The suppressive effect of C3 and the C3a-like fragments was not attributable to alterations in the kinetics of antibody production or to the induction of suppressor populations of lymphocytes or macrophages, which suggested the possibility of an action of C3a on B lymphocytes. However, the capacity of highly purified human C3a to inhibit the production of antibodies by mouse lymphocytes was not dependent on the suppression of B lymphocyte proliferation, a finding that implies that C3a may modify the function of one or more regulatory subsets of T lymphocytes (19). The reduced ability of human T lymphocytes to generate lymphokines in the presence of C3a or C3a(70–77), irrespective of the nature of the stimulus, and the removal or inactivation by solid-phase C3a(70–77) of the lymphocytes required for LIF generation provide direct evidence for functional modulation of T lymphocyte-dependent cellular immune phenomena by a product of the complement cascade.

Summary

Human C3a and the synthetic octapeptide C3a(70–77), which retains the activities of an anaphylatoxin, inhibit in a concentration-dependent manner the generation of leukocyte inhibitory factor (LIF) activity by human mononuclear leukocytes and T lymphocytes cultured with the mitogens phytohemagglutinin (PHA) or concanavalin A (Con A) or the antigen streptokinase-streptodornase (SK-SD). The generation of LIF activity was inhibited by 50% by 10^{-8} M C3a or C3a(70–77) with PHA or Con A as the stimulus, whereas a more than 10-fold higher concentration of C3a(70–77) than C3a was required to achieve the same level of suppression with SK-SD as the stimulus. Similar concentrations of C3a(70–77) inhibited to the same extent the migration of T lymphocytes stimulated by α-thioglycerol or Con A. Neither C3a nor C3a(70–77) altered significantly the uptake of [^{3}H]thymidine by human mononuclear cells exposed to PHA, Con A, or SK-SD. The capacity of C3a(70–77)-Sepharose, but not Sepharose alone, to adsorb or inactivate mononuclear leukocytes required for the generation of LIF activity established a direct interaction. Analysis of the lymphocytes in the effluent from C3a(70–77)-Sepharose columns, using monoclonal antibodies to surface antigens, showed a selective depletion of the helper/inducer population of lymphocytes. C3a might represent an important mediator of the functionally selective regulation of human T lymphocyte activities by the complement system.

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