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D J Beer, … , L J Rosenwasser, R E Rocklin

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Research Article

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Human Monocyte-derived Soluble Product(s) Has an Accessory Function in the Generation of Histamine- and Concanavalin A-induced Suppressor T cells

DENNIS J. BEER, CHARLES A. DINARELLO, LANNY J. ROSENWASSER, and ROSS E. ROCKLIN, *Divisions of Allergy and Experimental Medicine, Department of Medicine, Tufts University School of Medicine, New England Medical Center Hospital, Boston, Massachusetts 02111*

ABSTRACT We have analyzed the cellular interactions required for the generation of histamine- and concanavalin A (Con A)-induced suppressor T cells by employing a co-culture assay and techniques for fractionation of human blood mononuclear cells (PBMC). PBMC cultured in the presence of histamine (0.1 mM–1 mM) or Con A (20 μg/ml) for 24 h, mitomycin treated and subsequently combined with autologous mitogen-stimulated mononuclear cells, significantly suppressed a subsequent blastogenic response. PBMC fractionated over nylon wool columns and depleted of adherent cells and enriched for T cells (NWNA-T) were unable to generate suppressor activity. However, suppressor cell function by NWNA-T cells was reconstituted by the addition of autologous monocytes. In both the histamine and Con A suppressor systems, the requirement for monocytes in the activation process was enhanced by suspending the NWNA-T population in supernatants derived from allogeneic monocytes stimulated with heat-killed *Staphylococcus albus*.

These crude supernatants contained leukocytic pyrogen (LP) and lymphocyte activating factor (LAF). Sequential purification and separation of the crude supernatants using gel-filtration, immunoadsorption, and isoelectric focusing demonstrated that only those fractions containing LP and LAF were capable of reconstituting NWNA-T cell histamine and Con A-induced suppressor activity. Thus, these studies suggest that the accessory role of supernatants derived from activated monocytes in the generation of suppressor cells may be mediated by LP/LAF. Further studies are in progress to explore the mechanism by which soluble factors stimulate suppressor T cells.

INTRODUCTION

The participation of mononuclear phagocytes in T cell-mediated immune phenomena is well established. Several lines of investigation have shown that T cell suppressor activity is dependent on the presence of mononuclear phagocytes (1, 2). One suggested accessory function of monocytes/macrophages in T cell activation is the production of stimulatory molecules. Soluble products of monocytes/macrophages or monokines have been shown to be of major importance in the modulation of various in vitro immune phenomena (3).

Lymphocyte activating factor (LAF)[1] is a monokine that is involved in augmenting T lymphocyte proliferative responses to mitogen (4, 5). Early chemical characterization of human LAF showed similarities to human leukocytic pyrogen (LP), another monokine, which mediates fever (6–9). The possibility that LP could have an immunoregulatory role was first suggested by experiments in which purified human LP augmented the antigen induced proliferative response of murine T cells in a manner similar to that observed

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Dr. Beer is a Fellow of the Asthma and Allergy Foundation of America. Address all correspondence to Dr. Rocklin.

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[1] *Abbreviations used in this paper:* Con A, concanavalin A; HBSS, Hanks' balanced salt solution; HSF, histamine-induced suppressor factor; LAF, lymphocyte activating factor; LP, leukocytic pyrogen; NWNA-T, nylon wool nonadherent T enriched; PBMC, peripheral blood mononuclear cells; RPD, rabbit pyrogen dose; TC-199 HPS, medium TC-199 with penicillin, streptomycin, and Hepes.

with macrophage culture supernatant containing LAF activity (10). On the basis of biochemical similarities and functional characteristics, it was speculated that LP and LAF were closely related (10, 11). Other studies using LP derived from rabbit macrophages confirmed the initial observation that LP has LAF activity (12, 13). More recent studies have extended these initial findings by demonstrating the sequential copurification of LP and LAF activities (14, 15).

Because prior experiments (1, 2) have demonstrated the requirement of accessory mononuclear phagocytes for the induction of histamine and concanavalin A (Con A) activated human suppressor T cells, we investigated the role of soluble products derived from monocytes to support the generation of histamine and Con A-stimulated suppressor T cells. The results indicate that the requirement for monocytes during the activation process could be augmented by using crude adherent cell supernatants and that the active moiety in these supernatants copurify with LP and LAF.

METHODS

Animals. 6-wk-old female C57Bl 6/J (B6) mice, were used as thymocyte donors and were purchased from Jackson Laboratories, Bar Harbor, ME. New Zealand-derived white rabbits, weighing 2.5–3.0 kg were purchased from Associated Rabbit Industries, Avon, MA and were used to assay LP (8).

Purification of peripheral blood mononuclear cells and monocytes. Peripheral blood mononuclear cells were isolated from heparinized venous blood of healthy adult volunteers by density centrifugation on Lymphocyte Separation Medium (Bionetics, Kensington, MD) according to the method of Böyum (16). Cells were recovered from the interface, washed three times and suspended in medium TC-199 (Gibco Laboratories, Grand Island Biological Co., Grand Island, NY) supplemented with 100 U/ml of penicillin (P), 100 μg/ml of streptomycin (S) and 25 mM Hepes (H) buffer (TC-199 HPS).

To obtain a highly enriched population of blood monocytes, 75×10^6 mononuclear cells were suspended in 15 ml of TC-199 HPS (containing 15% AB human serum) and incubated on sterile glass petri dishes (15 × 100 mm) for 1–2 h at 37°C (10). Postincubation the plates were washed five times with warm media and the remaining adherent cells recovered by scraping the petri dish with a sterile rubber policeman and washing with chilled (4°C) medium. The latter cell population was highly enriched for monocytes as documented by esterase staining (17).

Preparation of nylon wool nonadherent T cell-enriched (NWNA-T) population. Nylon wool adherent cells were removed from peripheral blood mononuclear cells by a modification of the method of Julius et al. (18). 3 g of prewashed sterilized nylon wool were placed in a 30-cm^3 syringe. The nylon wool column was soaked with TC-199 HPS enriched with 10% human AB serum that had been prewarmed to 37°C. After this procedure, 100×10^6 mononuclear cells (suspended in 5 ml TC-199 HPS) were applied to the column and incubated at 37°C for 2 h. After incubation, the nonadherent cells were eluted with the same medium at a flow rate of 1 ml/min until 50 ml of cell suspension was collected. 25–40% of the cells were recovered in the eluate. This NWNA-T population was washed three times and resuspended in TC-199 HPS.

Generation of monocyte supernatants. Plateletpheresis byproducts were obtained from the Northeast Branch of the American Red Cross and contained large numbers of monocytes and lymphocytes. Mononuclear cells were separated by density centrifugation, washed in saline and suspended at 20×10^6/ml in Hanks' balanced salt solution (HBSS) (M.A. Bioproducts, Walkersville, MD) containing 2 U of heparin/ml, 100 U/ml penicillin G and 8 μg/ml gentamicin (6). Fresh AB serum (stored at −70°C) was added to make a final serum concentration of 5%. Heat killed *S. albus* were added to make a final bacteria-leukocyte ratio of 20:1 (6). After 60 min of incubation at 37°C in a shaking water bath, the cells were spun at 250 *g* for 5 min and resuspended at 4×10^6 cells/ml and added to flat-sided glass bottles at a *d* of 7×10^5 cells/cm^2 in fresh HBSS without serum. After 1 h incubation at 37°C, the bottles were shaken, the media and nonadherent cells removed and fresh HBSS added. Following a subsequent 18-h incubation at 37°C in a 5% CO_2-95% air atmosphere, the cell suspension was centrifuged at 2,200 *g* for 30 min.

The supernatant was removed and stored at 4°C for further use. Before use of this supernatant in a co-culture experiment, it was dialyzed against 100 vol of TC-199 (two changes) for 24 h and subsequently supplemented with penicillin (100 U/ml) and streptomycin (100 μg/ml). This material was assayed for pyrogen and LAF activity and was used to suspend the NWNA-T cell population. For certain experiments, control supernatants were generated from monocytes cultured in the absence of microorganisms and from heat killed *S. albus* incubated in medium without cells. These control supernatants were processed and assayed in an identical manner as the above supernatants.

Gel-filtration and immunoadsorption of human LP/LAF. Crude supernatants containing LP were concentrated in autoclaved standard dialysis tubing in front of a high-speed fan. Volumes were reduced to 1/20th the original amount. Concentrated crude monocyte supernatants were chromatographed over a Sephadex G-50 column and pooled fractions were analyzed for pyrogen and LAF activities as well as their ability to reconstitute the NWNA-T population suppressor cell response. The 15,000-mol wt fractions were pooled and then passed over a rabbit anti-human LP immunoadsorbent column (9). The breakthrough material, the citric acid eluate, and HBSS washes of the anti-human LP column were assayed for the activities mentioned above.

Isoelectric focusing. Isoelectric focusing was carried out using the 15,000-dalton fraction of LP/LAF isolated from four individual chromatographic separations of crude pyrogen over Sephadex G-50 columns. These were concentrated and dialyzed against 10 mM NaCl. 4 g of Sephadex G-75 (superfine) were added to 100 ml of the LP preparation containing 2% Ampholines (LKB Instruments, Bromma Sweden; ranges, 5–8 and 3.5–10). The slurry was poured onto a 20 × 10-cm glass plate; 1 M H_3PO_4 and 1 M NaOH were used to saturate anode and cathode filter paper contacts. The slurry was air dried using a fan at room temperature until a pastelike surface developed. 8 W of constant power (~600 V) was applied for 24 h using LKB Multiphor model 2117 maintained at 4°C. The thin-layer Sephadex was divided into 5 × 70-mm fractions, suspended in 2 ml water and the pH was determined. Each fraction was then washed with 10 ml of culture medium, centrifuged and the supernatant dialyzed against TC-199.

LP assay. The rabbit pyrogen assay was used to quan-

titate the amount of biologically active human LP in samples added to lymphocyte cultures using a two-point dose response. A rabbit pyrogen dose (RPD) is defined as the amount of LP needed to induce an elevation in rectal temperatures between 0.6 and 0.9°C (8).

LAF assay. LAF activity was determined by the enhancement of the B6 murine thymocyte mitogenic response to phytohemagglutinin (Burroughs Wellcome Co., Research Triangle Park, NC) as described previously (10, 14). Briefly, the thymus glands from 6-wk-old B6 mice were aseptically removed and teased apart between the frosted ends of two sterile glass slides in HBSS. Cells were washed vigorously and suspended in medium RPMI 1640 supplemented by 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.3 mg/ml fresh L-glutamine (Gibco Laboratories), 50 μM 2 mercaptoethanol (Eastman Kodak, Rochester, NY) 10 mM Hepes buffer and 5% fetal calf serum (Sterile Systems, Inc. Logan, UT). Into each well of a flat-bottom microtiter plate (Linbro Chemical Co., Hamden, CT), was aliquoted 5×10^5 thymocytes, phytohemagglutinin (1.0 μg/ml) and dilutions of monocyte supernates or fractions to obtain a final volume of 200 μl. These cultures were then incubated for 72 h at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. The wells were pulsed with 1 μCi/well of [^{3}H]thymidine (sp act 6.7 mCi/mM, New England Nuclear, Boston, MA) for the final 18 h of culture. The plates were then harvested using a multiple automated sample harvester and [^{3}H]thymidine incorporation was measured using liquid scintillation spectrometry. The results were reported as mean counts per minute for triplicate determinations and as Δ counts per minute above control.

In vitro co-culture suppressor cell assay. The in vitro co-culture technique for suppressor cell activation and assay by histamine (19) is similar to that reported for Con A (20). Blood mononuclear cells or NWNA-T cells cultured in crude supernatants or fractions were cultured at a concentration of 6×10^5 cells/ml in medium TC-199 HPS containing 15% normal AB human serum in the absence or presence of histamine (0.1–1 mM (Sigma Chemical Co.) or Con A (20 μg/ml) for 24 h at 37°C in a 5% CO_2-95% air atmosphere. Indicator cells from the same subject were cultured separately in the absence of any stimulant in medium TC-199 HPS containing 15% AB human serum. Following incubation, the control and suppressor cells were mitomycin-treated (50 μg/ml) (Sigma Chemical Co.) for 1 h at 37°C, washed three times in medium TC-199 HPS, combined with 1×10^6 indicator cells, and the final mixture of suppressor and indicator cells was suspended in 1 ml of medium TC-199 HPS containing 15% AB human serum. At the time of co-culture, the indicator cells were stimulated with 10 μg/ml of Con A and placed in microtiter plates (2×10^5 cells/well) for 3 d. 18 h before terminating the cultures, 1 μCi/well of [^{3}H]thymidine was added and its incorporation into cellular DNA was determined as described above.

The percent suppression of [^{3}H]thymidine uptake was determined by the following formula:

$$\% \text{ suppression} = \left(1 - \frac{\text{cpm in presence of histamine or Con A}}{\text{cpm in absence or histamine or Con A}}\right) \times 100.$$

The mean (±SD) counts per minute was determined from quadruplicate wells. The variability in the assay was <10%.

Statistical analysis. Statistical evaluation of differences in percent suppression in a given experimental protocol was determined by Student's *t* test for paired means.

RESULTS

Reconstitution of the NWNA-T population suppressor cell response by monocytes and by crude monocyte supernatants. The histamine and Con A-induced suppressor function of PBMC, NWNA-T cells, and NWNA-T cells supplemented with 10% monocyte or cultured in crude monocyte supernatants is summarized in Table I, where the data is presented both as the mean (±SD) counts per minute of quadruplicate cultures for each of three individual experiments as well as the mean (±SEM) percent suppression of the three experiments. Peripheral blood mononuclear cells (PBMC) contained 15–20% monocytes as determined by nonspecific esterase staining; the NWNA-T population contained <2% monocytes as judged by the same criterion. When the PBMC population was cultured with histamine or Con A for 24 h and then placed in co-culture with mitogen-stimulated autologous mononuclear cells, there was significant suppression of lymphocyte proliferation. In contrast, when the NWNA-T population was similarly precultured with histamine or Con A and then co-cultured with autologous mitogen-stimulated mononuclear cells, statistically less suppression was seen ($P < 0.05$). However if the NWNA-T population was supplemented with 10% monocytes or cultured in the presence of crude monocyte supernatant, the suppressor cell response to histamine and Con A was restored.

When either PBMC or the NWNA-T population were precultured with crude monocyte supernatants in the absence of histamine or Con A and then combined in co-culture with indicator cells, there was no evidence of "spontaneous" suppressor cell activity (data not shown). Thus, monocyte supernatants were not stimulatory by themselves and did not nonspecifically activate suppressor cells.

Gel filtration of crude monocyte supernatant over Sephadex G-50. Crude human monocyte supernatants were concentrated and applied to Sephadex G-50 (fine) column (165 × 5.6 cm). As depicted in Fig. 1A, two peaks of leukocytic pyrogen activity were detected at ~40,000 and 15,000 daltons. The fractions containing pyrogen activity as well as five other pooled fractions of varying molecular weights were isolated and dialyzed. These seven fractions were assayed for LAF activity and for their ability to reconstitute histamine and Con A-induced suppressor activity in the NWNA-T population. As shown in Fig. 1A, LAF activity coincided with pyrogenic activity appearing in both the 40,000- and 15,000-dalton species, i.e., fractions B and E, respectively.

Fig. 1B illustrates the ability of these same seven fractions to reconstitute the ligand-induced suppressor activity of the NWNA-T population. NWNA-T cells

TABLE I

*Reconstitution of NWNA-T Suppressor Cell Response by Monocytes or Crude Monocyte Supernatants**

Cell population	Suppressor stimulus	Experiment 1	Experiment 2	Experiment 3	Mean±SEM % suppression
PBMC	None	34,727±3,001‡	31,555±1,463	100,430±9,915	
	1 mM histamine	24,632±2,193 (29%)§	21,861±1,567 (31%)	78,555±5,009 (22%)	27±3‖
	20 μg/ml Con A	23,111±2,292 (33%)	24,861±2,376 (22%)	76,590±6,175 (23%)	26±4
NWNA-T	None	34,093±3,231	32,222±1,688	90,302±8,654	
	1 mM histamine	30,810±2,762 (10%)	32,301±1,009 (0%)	88,461±6,110 (2%)	4±3‖
	20 μg/ml Con A	29,686±1,843 (13%)	32,427±3,019 (0%)	86,782±7,192 (4%)	6±4
NWNA-T + Glass adherent cells¶	None	37,797±2,994	32,936±1,688	85,042±4,854	
NWNA-T + Glass adherent cells	1 mM histamine	29,917±2,524 (21%)	24,001±2,219 (27%)	68,485±7,001 (20%)	23±2
NWNA-T	20 μg/ml Con A	27,491±1,116 (27%)	26,100±2,471 (21%)	67,698±4,852 (21%)	23±2
NWNA-T + Crude monocyte supernatant	None	34,112±3,095	30,198±2,988	94,436±2,374	
NWNA-T + Crude monocyte supernatant	1 mM histamine	26,001±1,962 (24%)	24,018±1,812 (21%)	75,979±7,422 (20%)	22±1
NWNA-T + Crude monocyte supernatant	20 μg/ml Con A	23,798±2,284 (31%)	24,140±2,409 (20%)	74,358±5,112 (21%)	24±4

* PBMC or NWNA-T were precultured in the absence or presence of histamine (1 mM) or Con A (20 μg/ml) and subsequently co-cultured with autologous mitogen stimulated mononuclear cells. PBMC depleted of NWNA-T were unable to express suppressor activity when stimulated with histamine or Con A. Reconstitution of this T cell enriched population with 10% autologous monocytes in the form of glass adherent cells or the addition of crude monocyte supernatant restored histamine and Con A-induced suppressor activity.

‡ Mean±SD counts per minute of tritiated thymidine incorporation for quadruplicate cultures.

§ Percent suppression of tritiated thymidine incorporation.

‖ Mean percent suppression for both histamine and Con A are statistically different ($P < 0.05$).

¶ 1×10^5 NWNA-T cells + 1×10^4 glass adherent cells (>95% esterase positive) per well.

were cultured in each of the seven fractions in three separate experiments and data are presented as the mean (±SEM) percent suppression. The base-line histamine and Con A-induced suppressor responses in the PBMC and NWNA-T populations is shown on the far right in Fig. 1B. Only fractions containing both leukocytic pyrogen and LAF activities (B and E; 40,000 and 15,000 daltons, respectively) were able to restore suppressor activity to the NWNA-T population. When either PBMC or the NWNA-T populations were precultured with the active or inactive G-50 gel-filtration fractions in the absence of histamine or Con A and then combined in co-culture with mitogen-stimulated indicator cells, there was no evidence of "spontaneous" suppressor cell activity (data not shown).

The data in Table II indicate that the reconstitutive activity present in the 15,000-dalton fraction of the G-50 chromatograph of crude monocyte supernatant

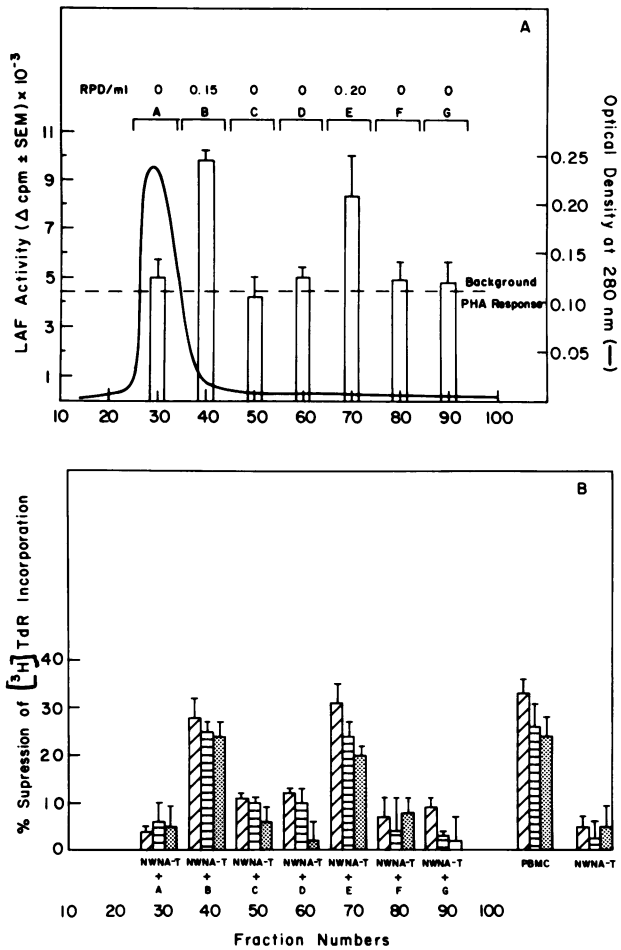

FIGURE 1 Sephadex G-50 gel filtration of crude monocyte supernatant showing coincident peaks (~ 40,000 and 15,000 daltons) of pyrogenicity, lymphocyte activating factor activity (A) and the ability to reconstitute histamine and Con A-induced suppressor activity of the NWNA-T population (B). ▨ 1 mM histamine; ▤, 0.1 mM histamine; □, 20 μg/ml Con A.

can be diluted at least 10-fold before its activity is significantly diminished, and cannot be detected after a 100-fold dilution. A 10-fold dilution of this activity ($\sim 10^{-2}$ RPD/ml), while clearly reconstitutive, is subpyrogenic in the rabbit fever assay. The LAF assay is still a sensitive test at 10^{-5} RPD/ml (14).

The effect of immobilized anti-LP or active fractions. The effect of passing partially purified leukocytic pyrogen over an anti-human LP immunoadsorbent is shown in Table III. The 15,000-dalton fractions obtained after gel-filtration, which contained all three biologic activities, were subsequently applied to an anti-human LP antibody column. The unbound effluent from this column did not contain significant amounts of LP and LAF activities and was unable to reconstitute histamine and Con A-induced suppressor

TABLE II
Dose Response of Partially Purified Leukocytic Pyrogen

	Suppressor stimulant		
	1 mM Histamine	0.1 mM Histamine	20 μg/ml Con A
	%	%	%
Control*	45‡	31	31
NWNA-T	0	−1	−2
NWNA-T + fraction E neat§	40	13	31
NWNA-T + fraction E 1:2	43	37	26
NWNA-T + fraction E 1:10	25	30	25
NWNA-T + fraction E 1:100	7	15	12

* Usual assay as outlined in Table I.
‡ Percent suppression of [³H]TDR incorporation.
§ Fraction E (Fig. 1a and 1b), 15,000 dalton fraction of G-50 chromatograph of crude monocyte supernatant (0.2 RPD/ml).

cell activity in the NWNA-T population. The citric acid eluate of the immunoadsorbent column possessed all three biologic activities. HBSS and citric acid washes of the anti-human LP antibody column prior to application of the 15,000-dalton fractions did not possess any of the aforementioned activities (data not shown).

Heat sensitivities of the biologic activities of partially purified LP/LAF. The effect of heating on the biologic activities of partially purified leukocytic pyrogen is shown in Table IV. Concentrated crude monocyte supernatant containing LP/LAF was passed over an immunoadsorbent rabbit anti-human LP antibody column, and the citric acid eluate subsequently chromatographed over a Sephadex G-50 column. When the 15,000-dalton species possessing pyrogenic, LAF, and suppressor cell reconstitution activities was heated at 70°C for 30 min, there was simultaneous loss of all three biologic activities.

Isoelectric focusing of partially purified LP/LAF. Four concentrated crude monocyte supernatants were individually chromatographed over a Sephadex G-50 and the 15,000-dalton fractions were pooled, concentrated, dialyzed, and subjected to isoelectric focusing. The results are summarized in Table V. 34 fractions were generated and all were assayed for pyrogenic and LAF activities. Two major peaks of pyrogen activity were found, having a pI of 6.73 (6.61–6.84) and 4.95 (4.87–5.03). Two major peaks of LAF activity were also detected and appeared in the same fractions containing LP activity.

Table V also shows the relationship between LP and LAF activities and the ability of several pooled fractions to reconstitute the histamine and Con A-induced suppressor activity of the NWNA-T population. Only the fractions containing LP and LAF activities were

TABLE III
Immunoadsorbance of Activities by Rabbit Anti-human Leukocytic Pyrogen

Immunoadsorbed supernatant	Pyrogenic*	LAF‡	Suppressor stimulant§		
			1 mM Histamine	0.1 mM Histamine	20 μg/ml Con A
			%	%	%
Unadsorbed partially purified leukocytic pyrogen	0.2 RPD/ml	16,931	30	20	31
Column effluent	0.0 RPD	3,195	1	2	5
Citric acid eluate	0.15 RPD/ml	12,064	31	24	23

* Crude monocyte supernatant chromatographed over a Sephadex G-50 column and the resulting 15,000-dalton fraction was then passed over an immunoadsorbant rabbit anti-human LP antibody column.
‡ Expressed in rabbit pyrogen doses (see Methods).
§ Enhancement of B6 murine thymocyte mitogenic response to PHA expressed as Δcounts per minute above base-line response. Media control PHA response: Δcpm = 2,591.
‖ Percent suppression of a mitogen stimulated proliferative response by a NWNA-T population activated by either histamine (0.1–1 mM) or Con A (20 μg/ml) in the presence of each supernatant.

able to restore suppressor activity to the NWNA-T population. As before, these fractions were not stimulatory by themselves and did not activate suppressor cells within the PBMC or NWNA-T populations without another signal provided either by histamine or Con A.

TABLE IV
*Effect of Heating on the Biologic Activities of Partially Purified Leukocytic Pyrogen**

	Unheated	70°C Heating	% Loss of activity
Pyrogenicity in rabbits‡	0.2 RPD/ml	0	100
LAF§	14,745	4,576	92
Suppressor cell activity at 1 mM histamine‖	30%	9%	70
Suppressor cell activity at 20 μg/ml Con A¶	21%	1%	95

* Concentrated crude monocyte supernatant was passed over a rabbit anti-human LP-antibody immunoadsorbant column and the citric acid eluate subsequently applied to a Sephadex G-50 gel-filtration column. The resulting 15,000-dalton species possessing pyrogenic, LAF, and suppressor cell reconstitution activities was heated at 70°C for 1 h.
‡ Expressed as RPD/10 ml with 1 RPD representing that amount of pyrogen required to cause peak eluation in rectal temperature between 0.6 and 0.9°C.
§ Enhancement of B6 murine thymocyte mitogenic response to PHA expressed as Δcounts per minute above base-line response. Media control PHA response: Δcpm = 3,707.
‖ Percent suppression of a mitogen stimulated proliferative response by a NWNA-T population activated by 1 mM histamine in the presence of partially purified LP.
¶ Percent suppression of a mitogen-stimulated proliferative response by a NWNA-T population activated by 20 μg/ml Con A in the presence of partially purified LP.

DISCUSSION

Investigators (1, 2) have been able to partially characterize the cellular interactions involved in the activation of suppressor cells and the expression of their function using a co-culture assay and techniques for fractionation of human blood mononuclear cells. The afferent limb of suppressor activity involves several steps linked by T cell-monocyte cooperation. Initial studies clearly demonstrated that the monocyte was required as an accessory cell for the triggering of T suppressor cells by histamine (21). Evidence for this conclusion was provided by the observation that PBMC depleted of adherent cells were unable to generate or express suppressor activity. However, suppressor cell function by the NWNA-T population could be reconstituted by the addition of monocytes in the form of glass-adherent cells. In both the histamine and Con A suppressor systems, the requirement for normal numbers of monocytes during the activation process could be augmented by using crude supernatants derived from activated monocytes (1×10^6/ml).

The present investigation was designed to define the active moieties present in crude monocyte supernatants that participate in the generation of suppressor T cells by histamine and Con A. The data in this report confirm the previous copurification of LP and LAF activities (11, 14) and now demonstrate that these monokine-rich fractions are a required accessory factor in the activation of T suppressor cells by both histamine and Con A.

When crude human monocyte supernatants were applied to a Sephadex G-50, two fractions of ~40,000 and 15,000 daltons were identified as being capable of restoring histamine and Con A-induced suppressor activity to the monocyte-depleted T cell-enriched pop-

TABLE V

Isoelectric Focusing of Partially Purified Leukocytic Pyrogen

	Pyrogenicity*	LAF‡	Suppressor stimulant§		
pH	ΔT (°C)	Δcpm	1 mM Histamine	0.1 mM Histamine	20 μg/ml Con A
			%	%	%
8.21–8.08	0.0, 0.0	1,758	6	2	2
6.84–6.61	0.7, 0.9	7,099	22	20	28
5.54–5.32	0.0, 0.0	2,125	3	1	1
5.03–4.87	0.4, 0.5	6,424	26	22	20
3.98–3.88	0.0, 0.0	752	3	4	0

Concentrated crude monocyte supernatant was chromatographed over a Sephadex G-50 gel-filtration and the 15,000-dalton fractions were subjected to isoelectric focusing.

* Peak fever (°C) over baseline in individual rabbits injected with 5 ml of each fraction.

‡ B6 murine thymocyte mitogenic response to PHA expressed as counts per minute. Base line PHA response for media control was cpm = 1,917.

§ Percent suppression of a mitogen-stimulated proliferative response by a NWNA-T population activated by either histamine (0.1–1 mM) or Con A (20 μg/ml) in the presence of each of the above listed fractions.

ulation (Fig. 1B). These fractions did not activate spontaneous suppressor activity in either the PBMC or NWNA-T populations. Pyrogenicity and LAF were coincident at these molecular weights and were not observed in the other molecular weight fractions. Previous studies (10–12, 14) have shown similar molecular weights for LP/LAF.

When the 15,000-dalton fraction, which possessed all three biologic activities, was subsequently applied to a rabbit anti-human LP Sepharose 4B immunoadsorbent, all three activities were absent from the effluent (Table III). The eluate from subsequent washing of the immunoadsorbent with citric acid possessed all three biologic activities. The rabbit antibody used in this study has been described previously (7, 9). It was produced using prolonged immunizations with crude human monocyte supernates and the antiserum was subsequently purified using several immobilized monocyte and serum derived proteins without LP activity. The purification of the crude antiserum using immobilized antigens was carried out with several passages until no precipitating bands were observed on immunodiffusion. Despite several passages, anti-LP activity was not diminished and the resulting anti-LP was shown to be highly selective in the purification of crude LP (9). In the present study, LP obtained from gel-filtration and subsequently eluted from the anti-immunoadsorbent represents a significant purification and characterization step. Thus, the coelution of LP with the soluble accessory factor for suppressor T cell activation, and LAF supports the concept that this monokine augments human T cell function as well as enhancing the activity of murine thymocytes. These separation studies were extended to include charge differences by isoelectric focusing. Previous studies established that human LP was comprised of two major isoelectric points at ~pH 5.1 and 6.8 (6). In the present studies, these two pI were also shown to contain the soluble accessory factor for suppressor T cell activation as well as LP activity. In addition, the active fractions also had significant LAF activity.

Although LP copurifies with LAF and the soluble accessory factor for suppressor T cell activation, these studies do not involve the use of a documented homogenous preparation of human LP or LAF using a criteria such as homologous N-terminal amino acid sequence data. Because of difficulties in recovering sufficient LP or LAF during purification procedures, such preparations are currently not available. However, it is of interest that significant loss of fever, LAF, and suppressor cell reconstitution was noted when monokine containing supernatant was treated at 70°C for 1 h (Table IV). Despite the lack of formal proof at present, we propose that molecules capable of inducing fever may also act as an accessory factor in the activation of lymphocytes in vitro. Studies from this and other laboratories have suggested that in addition to causing fever and supporting lymphocyte activation, human LP/LAF also stimulates neutrophil-specific granule release (22), neutrophil superoxide production (23) and the synthesis of acute phase proteins such as serum amyloid A protein (15, 24). Finally, the dose-response data indicate that although reconstitution of suppressor cell activity appears to be a more sensitive indicator of LP activity than the rabbit fever assay, it is still less sensitive than the thymocyte proliferation assay (LAF).

The present experiments suggest that one of the ways in which monocytes facilitate histamine and Con A induction of suppressor T cells is by providing mol-

ecules that maintain an appropriate environment in which T cells can respond appropriately to given signals. As determined by biochemical purification including the use of a specific immunoadsorbent, these stimulatory molecules appear to be the previously identified mediators LP/LAF/interleukin I (IL-1). The rabbit antiserum used in these studies, while relatively specific, is not of monoclonal origin. Therefore, the possibility is not excluded that antibodies are present to LP, LAF, and T suppressor cell reconstituting factor. This is the first demonstration of an IL-1 or monokine requirement in the activation of human suppressor cells. Our findings do not mitigate against the possibility that monocytes also function by presenting the ligand to the T lymphocyte. Even after enriching the suppressor population for T cells, 1–2% monocytes remain and can conceivably function in this capacity. In this setting then, the addition of monocyte supernatant containing LP/LAF may amplify the accessory function of the residual mononuclear phagocytes.

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