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Inhibition of leukocyte chemotaxis by serum factor in diabetes mellitus: Selective depression of cell responses mediated by complement-derived chemoattractants

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Abstract

Rat neutrophil chemotactic responses to N-formyl-methionyl-leucyl-phenylalanine (FMLP), leukotriene (LT) B_4, and lipopolysaccharide-activated serum (LPS-AS) were quantitatively assessed using the micropore filter system. Cells were suspended in either normal or diabetic rat serum for testing. Diabetic donor serum did not affect migration of neutrophils in a concentration gradient of the synthetic chemotactic agents. In contrast, the migratory responses to LPS-AS were significantly less than normal in this circumstance. Summation of effects was observed when FMLP and LPS-AS, or LTB_4 and LPS-AS were simultaneously added to the test chamber, with cells suspended in normal serum. Suspended in diabetic rat serum neutrophils responded normally to the synthetic chemoattractants but the response to the activated serum was blocked. Cells previously incubated in the presence of diabetic donor serum then transferred to a culture medium for testing, presented reduced migratory responses to LPS-AS. Supramaximal, inhibitory concentrations of FMLP and LTB_4, did not influence the response of neutrophils to LPS-AS. *In vivo*, suppression of cellular emigration to an inflamed area was observed from the early stages of the diabetic state. The inhibitory activity of chemotaxis in diabetes mellitus was previously reported to be associated with a protein factor in plasma of the animals. It is suggested that the inhibitory factor of chemotaxis in diabetes mellitus interacts with neutrophil receptors for complement-derived chemoattractants to induce blockade of cell-oriented locomotion either *in vitro* or *in vivo*.

Introduction

An enhanced susceptibility to infection is thought to occur in a poorly controlled diabetic state. Critical evaluations of the topic suggest that infection is more serious and is possibly more difficult to eradicate in the diabetic host [1–3]. In addition, there is a recognized group of rare infections where the incidence is definitely increased in diabetes mellitus and which may be confined almost entirely to the diabetic patient [2]. At least in part this condition might result from the impaired capacity to mount an inflammatory reaction [4–6], from functional changes in the behavior of microvessels [7], and from decreased responsiveness of the endothelial cell to endogenous vasoactive agents [8–10]. Furthermore, investigations on chemotaxis of polymorphonuclear leukocytes from diabetic subjects show that, while there are a few negative results, defective responses of the cells in the presence of a chemoattractant gradient can occur. The defect in chemotaxis is not correlated with plasma insulin and plasma glucose levels, serum carbon dioxide, or blood urea nitrogen values [11–14].

Recent observations indicate that the early local exudative cellular reaction in an inflammatory lesion is impaired in alloxan-induced diabetic rats

due to a reduced migration of neutrophils to the inflamed area. Neutrophils, however, are capable of moving from reserve compartments into blood in these animals, as much as in controls. Furthermore, an intrinsic cellular defect does not occur, because leukocytes obtained from diabetic animals are not devoid of chemotactic responsiveness when suspended in culture medium or normal serum. Suspended in the corresponding "diabetic" serum, blockade of chemotaxis to lipopolysaccharide-activated serum is observed. Increasing concentrations of diabetic serum added to a suspension containing neutrophils from normal donors progressively inhibit the response of the cells to the activated serum. Hyperglicemia alone, or hyperosmolality secondary to hyperglicemia, the presence of ketone bodies, malnutrition, or a direct effect of alloxan do not explain the results. In addition, the capacity to generate chemotactic agents remains intact in serum of diabetic animals. The defect appears to be due to the presence of an inhibitory factor in plasma which is heat labile (56 °C), is destroyed by incubation with trypsin, and is retained after dialysis with 10000-Mr retention dialysis tubing. Pretreatment of the animals with insulin results in the recovery of the chemotactic response, either *in vivo* or *in vitro* [15].

The present study was undertaken to investigate the influence of diabetic rat sera on the migratory response of neutrophils to various chemotactic agents. This was done in the attempt to elucidate the mechanism of action of the inhibitory factor occurring in diabetes mellitus. The data to be presented suggest that the inhibition is restricted to complement-derived chemotactic fractions, that blockade or saturation of receptors to synthetic chemoattractants do not affect chemotaxis to lipopolysaccharide-activated serum, and that the inhibitory activity develops at an early stage of the diabetic state.

Methods

Animals

Male Wistar rats weighing 200–220 g at the beginning of the experiments, and supplied with food and water *ad libitum*, were used.

Chemotactic agents

Lipopolysaccharide from *Escherichia coli* (LPS, serotype 026:B6, Sigma Chemical Company, St. Louis, MO) was incubated in rat serum (65 µg/ml) for 30 minutes at 37 °C. Varying concentrations of LPS-activated sera (LPS-AS) were obtained with dilution in Hanks balanced salt solution (HBSS). N-formyl-methionyl-leucyl-phenylalanine (FMLP, Sigma Chemical Company) and its specific antagonist tert-butyloxycarbonyl-phenylalanyl-leucyl-phenylalanyl-leucyl-phenylalanine (t-BOC-Phe-Leu-Phe-Leu-Phe, Sigma Chemical Company) were dissolved in dimethyl sulfoxide (DMSO, Aldrich Chemical Company, Milwaukee, WI) and stored at −20 °C. For use, the peptides were diluted in HBSS. The final concentration of DMSO employed did not detectably influence polymorphonuclear leukocyte function. Leukotriene (LT) B_4 (Sigma Chemical Company) was aliquoted and stored at −20 °C in absolute ethanol. At the moment of use, the free acid solution was dried under nitrogen and dilutions were made with HBSS.

Leukocyte suspension

Rat polymorphonuclear leukocytes (neutrophils) were obtained 12 h after the intraperitoneal injection of 20 ml of a 1% oyster glycogen (Type II, Sigma Chemical Company) solution in physiological saline. The animals were anesthetized with ether and the cells collected by rinsing the abdominal cavity with an adequate volume of physiological saline containing 1 U/ml heparin (Roche Laboratories, São Paulo, SP). Cells were centrifuged at 450 *g* for 7 minutes, and the contaminating erytrocytes lysed by the addition of hypotonic saline. The cells were then washed twice in HBSS and finally suspended in homologous sera obtained from normal or diabetic animals for chemotactic assays. Cell viability was confirmed by the trypan blue exclusion method.

Leukocyte chemotaxis assay

The migratory assay employed a modified Boyden chamber as described previously [16]. In brief, 0.4 ml aliquots of cell suspensions containing 1.5×10^6 neutrophils were added to the upper compartment of the modified Boyden chamber

separated from the chemotactic agent(s) in the lower compartment by a 13 mm diameter filter of 8 μm pore size (Millipore Industries, São Paulo, SP). HBSS was substituted for the chemotactic agent to measure control migration. In studies where summation of effects was investigated, migratory responses of the cells were assessed in the presence of submaximal concentrations of two chemotactic agents. In order to determine whether saturation of receptors to FMLP or LTB_4 influenced the response to LPS-AS, supramaximal, inhibitory concentrations of the former were placed above and below filter, whereas effective concentrations of the activated serum were added to the lower compartment of the chamber. Interaction of effects between FMLP and its competitive antagonist, t-BOC-Phe-Leu-Phe-Leu-Phe, was investigated by exposing the cells simultaneously to the antagonist (upper compartment) and the agonist (lower compartment). The loaded chambers were incubated in humidified air at 37 °C for 60 minutes, with the exception of one series of experiments in which incubation time varied from 10 to 60 minutes. At the end of the incubation the top fluid was aspirated and filters were removed for fixation and staining. With the micrometer of the fine adjustment of the microscope, the distance was measured from the top of filter to the farthest plane of focus still containing two cells with a ×40 objective [17]. Duplicate chambers were always run. Five fields were counted and averaged for each filter.

Leukocyte infiltration studies in vivo

To induce the accumulation of an inflammatory exudate into the pleural cavity [18], 50 μg carrageenan sodium salt, 60000–100000-Mr (Marine Colloids, Springfield, NJ) was dissolved in phosphate buffered saline (PBS) and injected in a standard volume of 0.1 ml into animals under light ether anesthesia. Four hours later, the animals were reanesthetized with ether, the chest wall opened, and the inflammatory exudate withdrawn after washing both pleural spaces with 2 ml PBS containing 1 U/ml heparin. Total and differential leukocyte counts were taken in the exudate. Total leukocyte counts were made in an automated cell counter. Cells in the exudate were washed three times in heparinized PBS before they were counted. Differential leukocyte counts were carried out in stained exudate films.

Induction of diabetes mellitus

Diabetes mellitus was induced with an intravenous injection of 40 mg/kg alloxan (Sigma Chemical Company) dissolved in physiological saline. Control rats were sham injected with physiological saline alone. The presence of diabetes was verified at the moment of use by blood glucose concentrations above 200 mg/dl, determined with a blood glucose monitor in samples obtained from the cut tip of the tail. At death a sample of blood was collected from the abdominal aorta while the animals were under ether anesthesia. In this sample serum immunoreactive insulin was measured by standardized radioimmunoassay [19], and the presence of ketone bodies was qualitatively assessed with the aid of reagent strips (Miles Laboratories, São Paulo, SP) in a fashion similar to the testing of urine. Insulin levels ranged between 2–18 μU/ml and 22–35 μU/ml in alloxan-induced diabetic rats and control animals, respectively. Ketone bodies were present in 80% of the diabetic rats.

Data analysis

Means and standard errors of means of all data are presented and were compared by Student's *t*-test with significant probability levels of less than 0.05.

Results

Neutrophils collected from the peritoneal cavity of normal rats, were washed and suspended in either normal or diabetic rat serum. Animals were rendered diabetic by the injection of alloxan 30 days before. Chemotaxis to FMLP, LTB_4 and LPS-AS was then assessed during 60 minutes at 37 °C with the aid of micropore filters. The results of these experiments are presented in Fig. 1. Diabetic rat serum did not affect migration of neutrophils in a concentration gradient of the synthetic chemotactic agents. Equivalent mean effective concentrations (EC_{50}), i.e. the concentration of the chemotactic agent giving 50% of the maximum activity, were estimated from concentration-effect curves constructed for FMLP and LTB_4 when tests were performed in the absence or presence of diabetic rat serum. In both circumstances, values were $2.7 \pm 0.3 \times 10^{-9}\ M$ and $1.5 \pm 0.3 \times 10^{-7}\ M$ for FMLP and LTB_4, respectively. In contrast, neutrophils suspended in diabetic rat serum exhibited

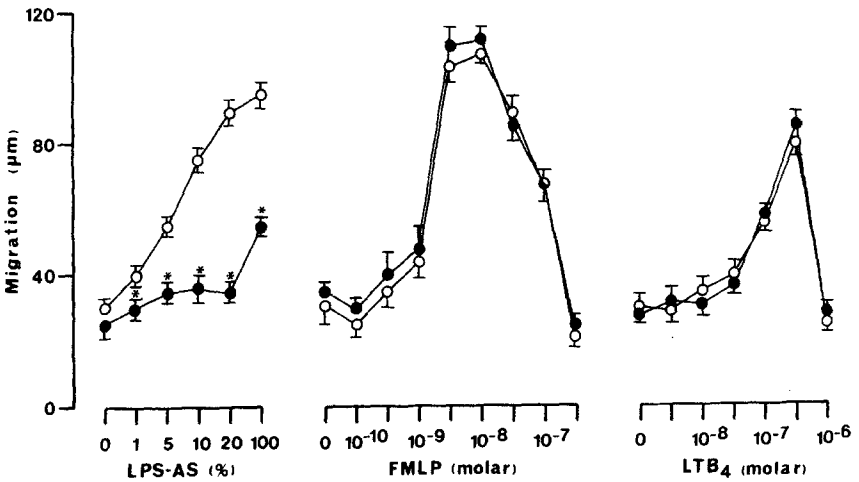

Figure 1
Chemotactic responses of neutrophils assessed by the micropore filter system. Cells were collected from the peritoneal cavity of normal rats and suspended in normal (o) or diabetic rat serum (•). Chemoattractants used were FMLP, LTB_4 and LPS-AS in HBSS. Chemotactic chambers were incubated at 37 °C for 1 h. Each point represents the mean ± SE of six separate experiments, each done in duplicate. * Differences from corresponding controls at $p < 0.05$.

Table 1
Chemotactic responses of neutrophils to LPS-AS in the presence of supramaximal, inhibitory concentrations of FMLP and LTB_4.

Upper compartment (cells suspended in)	Lower compartment	Migration into filter (µm)
Normal serum	FMLP (5×10^{-7} *M*)	20.1±1.1
Normal serum + FMLP (5×10^{-7} *M*)	FMLP (5×10^{-7} *M*)	22.1±1.0
Normal serum + FMLP (5×10^{-7} *M*)	FMLP (5×10^{-7} *M*) + LPS-AS (10% dilution)	68.7±2.1 *
Normal serum	LTB_4 (6×10^{-7} *M*)	22.0±1.9
Normal serum + LTB_4 (6×10^{-7} *M*)	LTB_4 (6×10^{-7} *M*)	28.2±2.0
Normal serum + LTB_4 (6×10^{-7} *M*)	LTB_4 (6×10^{-7} *M*) + LPS-AS (10% dilution)	74.8±1.0 *

Rat peritoneal neutrophils were used. Chemotactic chambers were incubated at 37 °C for 1 h. Control value for migration in response to LPS-AS (10% dilution) alone was 74.0±2.2 µm, as shown in Fig. 1. Each value represents the mean ± SE of three separate experiments, each done in duplicate. * $p < 0.05$ compared with corresponding value in the absence of LPS-AS.

migratory responses to LPS-AS which were significantly less than normal in the whole range of dilutions of the chemotactic material. The findings suggested that a remarkable degree of specificity of the receptor substance controlling the chemotactic response to FMLP, LTB_4 and LPS-AS occurred in terms of inhibition of this response by diabetic rat serum.

To further investigate these differences, effective concentrations of the chemoattractants were assayed either individually or in association, in the presence or absence of diabetic rat serum. Summation of effects was observed when FMLP and LPS-AS, or LTB_4 and LPS-AS were simultaneously added to the micropore filter system, provided the cells were suspended in normal serum. Suspended in diabetic donor serum, neutrophils responded in a normal fashion to the synthetic chemoattractants, but the response to LPS-AS was blocked and, consequently, no summation of effects occurred. Results are summarized in Fig. 2.

In order to ensure that the migratory responses to the different chemoattractants in the present conditions were governed by distinct receptor populations, supramaximal, inhibitory concentrations of FMLP and LTB_4 were added to the test chamber aiming at producing saturation of the corresponding receptors. Since supramaximal concentrations

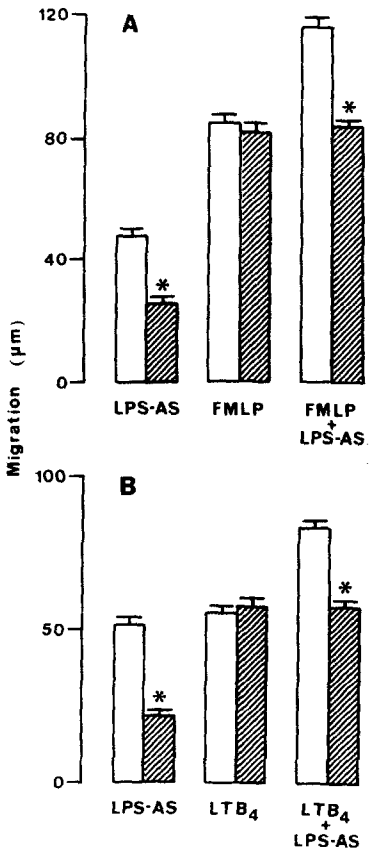

Figure 2
Chemotactic responses of neutrophils assessed by the micropore filter system. Cells were collected from the peritoneal cavity of normal rats and suspended in normal (open areas) or diabetic rat serum (hatched areas). Chemoattractants used were LPS-AS (5% dilution) and FMLP (2.5×10^{-9} *M*) in A, or LPS-AS (5% dilution) and LTB_4 (1.5×10^{-7} *M*) in B, tested either alone or in association. Chemotactic chambers were incubated at 37 °C for 1 h. Each point represents the mean ± SE of five separate experiments, each done in duplicate. * Differences from corresponding values obtained for cells suspended in normal serum at $p < 0.05$.

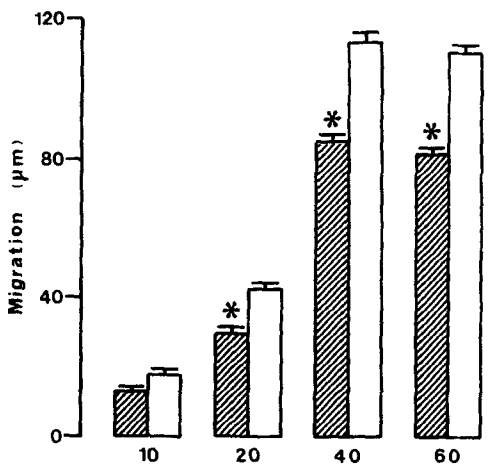

Figure 3
Chemotactic responses of neutrophils assessed by the micropore filter system. Cells were collected from the peritoneal cavity of normal rats, suspended in normal (open areas) or diabetic rat serum (hatched areas) then incubated at 37 °C for 1 h. Cell pellets, obtained by centrifugation, were immediately resuspended in HBSS and chemotactic responses to LPS-AS (10% dilution) estimated during varying intervals of time at 37 °C. Each point represents the mean ± SE of five separate experiments, each done in duplicate. * Differences from corresponding controls at $p < 0.05$.

Table 2
Effect of t-BOC-Phe-Leu-Phe-Leu-Phe on the chemotactic responses of neutrophils to FMLP and LPS-AS.

Chemoattractant	Migration into filter (µm)	
	t-BOC-Phe-Leu-Phe-Leu-Phe	
	Absent	Present
FMLP (1.0×10^{-9} *M*)	33.0 ± 2.6	24.5 ± 2.9 *
FMLP (2.5×10^{-9} *M*)	74.0 ± 6.2	38.0 ± 3.1 *
FMLP (5.0×10^{-9} *M*)	115.5 ± 3.4	70.0 ± 4.3 *
LPS-AS (10% dilution)	76.2 ± 3.5	74.0 ± 1.2

Rat peritoneal neutrophils suspended in normal homologous serum were used. Chemotactic chambers were incubated at 37 °C for 1 h. Each value represents the mean ± SE of three separate experiments, each done in duplicate. * $p < 0.05$ compared with corresponding value in the absence of t-BOC-Phe-Leu-Phe-Leu-Phe.

of the chemotactic agents were present above and below filter, dissociation from receptors was unlikely to occur. Chemotaxis to LPS-AS was then assessed, with cell suspensions prepared in normal rat serum, and showed that the inhibitory concentrations of the synthetic chemotactic agents did not influence the response to LPS-AS. Results are presented in Table 1. Another series of experiments indicated that when t-BOC-Phe-Leu-Phe-Leu-Phe, a specific antagonist of FMLP, was added to the cell suspension, inhibition of the response to the chemotactic peptide occurred, whereas the response to LPS-AS remained unaffected (Table 2). Accordingly, blockade of cell receptors to FMLP or LTB_4, using either supramaximal concentrations of the agonists, or effective concentrations of a corresponding antagonist, did not interfere with chemotaxis to LPS-AS.

Table 3
Cell migration to the pleural cavity of alloxan-induced diabetic rats and matching controls during a 4-hour carrageenan pleurisy.

Animals	Serum insulin	Plasma glucose	Cells $\times 10^6$ in inflammatory exudate		*n*
	(μU/ml)	(mg/dl)	Polymorphic	Mononuclear	
Diabetic (1 day)	12.4±1.6*	360±13*	35.82±3.84	7.03±0.46	13
Matching controls	37.0±2.1	88± 5	32.26±3.75	8.49±0.94	8
Diabetic (3 days)	12.8±1.5*	327±22*	22.90±3.02*	5.65±0.91	12
Matching controls	38.4±1.9	98± 4	32.90±2.72	6.55±0.56	8
Diabetic (30 days)	10.1±1.9*	278±42*	19.30±2.90*	5.87±0.47	13
Matching controls	38.1±2.0	94± 2	36.72±3.01	6.31±0.47	8

Animals were rendered diabetic by the injection of alloxan 1, 3 or 30 days before. The inflammatory exudate was obtained from the pleural cavity 4 h after the injection of 50 µg carrageenan. Each value represents the mean ± SE; *n* = number of animals. * $p < 0.05$ compared with values in matching controls.

In the attempt to analyse the mechanism responsible for the inhibitory activity of chemotaxis in diabetes mellitus, cells were incubated with either normal or diabetic rat serum for 1 h at 37 °C then centrifuged and the cell pellets immediately resuspended in HBSS. Responses to LPS-AS were estimated allowing cells to migrate during varying intervals, from 10 to 60 minutes. Results are presented in Fig. 3 and show that, relative to controls, a reduced migration occurred during each interval for cells previously incubated with diabetic donor serum. Therefore, binding of serum factors to the cells, or a long-lasting interference with post-receptor events appeared to occur in diabetes mellitus leading to suppression of neutrophil responses to serum-derived chemotactic agents.

The inhibitory activity of chemotaxis presently investigated was detected shortly after the onset of the diabetic state, as shown by *in vivo* experiments. The total number of cells present in the pleural cavity of the animals 4 h after initiation of a carrageenan-induced pleurisy was markedly decreased in diabetic animals compared with that of controls. The effect was observed from the third day of alloxan administration. Because the absolute number of mononuclear leukocytes in the inflammatory exudate was identical in normal and diabetic animals, being virtually indistinguishable from the number of mononuclear cells normally found in the pleural cavity of rats [20], and because the presence of eosinophils in the exudate was extremely rare, a decreased migration of neutrophils accounted for the reduction in the number of cells entering the pleural cavity of diabetic rats during the 4-hour carrageenan pleurisy. Comparable results were obtained in ketotic and non-ketotic diabetic animals. Results are summarized in Table 3.

Discussion

Our findings indicate that neutrophil migratory responses *in vitro* to FMLP and LTB_4 were not affected by the presence of diabetic rat serum whereas locomotion induced by LPS-AS was markedly inhibited. Concentration-effect curves constructed from the response of the cells to the different active materials, in the absence or presence of diabetic donor serum, showed that a blockade of neutrophil migration over the entire range of dilutions of LPS-AS occurred in the latter condition. In contrast, analogous curves were obtained with the use of the synthetic substances in both circumstances. FMLP and LTB_4 are truly chemotactic for polymorphonuclear leukocytes [21–24]. Activation of the complement system results in the rapid appearance of chemotactic activity [25–28]. Bacterial lipopolysaccharides have been demonstrated to activate both the classical and the alternative pathways of complement by a mechanism that does not require antibody to the LPS molecules [29–33]. Upon interaction of LPS with fresh sera from various species a chemotactic factor for polymorphonuclear leukocytes is generated. The generation of the chemotactic factor by LPS is dependent on an intact complement system involving at least the fifth complement component [34]. Distinct subsets of chemotactic receptors mediate the response of neutrophils to FMLP, LTB_4

and complement-derived chemotaxins [35–41]. This observation was corroborated by the present findings showing that supramaximal inhibitory concentrations of FMLP and LTB_4 or the presence of a specific antagonist of FMLP in the test chamber did not influence the chemotactic response of neutrophils to LPS-AS. Accordingly, the inhibitory activity of chemotaxis occurring in alloxan-induced diabetic rat serum appears to specifically block the response of the cells to complement-derived chemoattractants. This activity was shown to be heat labile (56 °C) destroyed by incubation with trypsin, and retained after dialysis with 10000-Mr retention dialysis tubing, thereby providing good evidence that the inhibition is associated with a protein factor in diabetic rat plasma [15]. It is plausible, therefore, that the inhibitory factor interacts with the C5a receptor on the surface of neutrophils to block chemotaxis in diabetes mellitus. Two lines of evidence supported this suggestion. First, summation of effects was observed when FMLP and LPS-AS, or LTB_4 and LPS-AS were simultaneously added to the test chamber, with cells suspended in normal donor serum. Suspended in diabetic donor serum, however, neutrophils responded normally to the synthetic chemoattractants, but the response to LPS-AS was blocked and no summation of effects occurred. Second, cells previously incubated in diabetic rat serum then suspended in HBSS for testing, always presented reduced migratory responses to LPS-AS, relative to controls, suggesting that the inhibitory factor binds to receptor structures on the neutrophil surface or leads to a long-lasting interference with post-receptor events.

Inhibition of chemotaxis is an early event in diabetes mellitus as indicated by *in vivo* experiments. Migration of neutrophils to an inflamed area was impaired already three days after the onset of the diabetic state, the inhibition persisting throughout the experimental period. The reduced cellular emigration to the inflammatory focus and the apparent specificity of the inhibitory factor of chemotaxis in diabetes provide good evidence that complement-derived chemoattractants are relevant for cell-oriented locomotion *in vivo* and that suppression of their effects might result in a defective host defense against infection.

Neutrophil chemotactic responsiveness is reported to be impaired in thermally injured patients [42–44] and to be inversely related to the extent of burn trauma [45]. Suppression of chemotaxis in diabetes mellitus might be another example of a cell-directed inhibitory activity occurring in a condition of poorly controlled metabolic derangement. At least in part, the occurrence of an inhibitor of neutrophil chemotaxis might account for the enhanced susceptibility to opportunistic infections in the diabetic host.

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References

[1] J. A. Savin, *Bacterial infections in diabetes mellitus.* Br. J. Dermatol. *91,* 481–487 (1974).

[2] J. G. Larkin, B. M. Frier and J. Ireland. *Diabetes mellitus and infection.* Postgrad. Med. J. *61,* 233–237 (1985).

[3] J. Garcia-Leme, *Hormones and Inflammation,* 238 pp, CRC Press, Boca Raton 1989.

[4] J. Garcia-Leme, L. Hamamura, R. H. Migliorini and M. P. Leite, *Influence of diabetes upon the inflammatory response of the rat: a pharmacological analysis.* Eur. J. Pharmacol. *23,* 74–81 (1973).

[5] J. Garcia-Leme, G. M. Bohm, R. H. Migliorini and M. Z. A. Souza, *Possible participation of insulin in the control of vascular permeability.* Eur. J. Pharmacol. *29,* 298–306 (1974).

[6] M. A. S. Llorach, G. M. Bohm and J. Garcia-Leme, *Decreased vascular reactions to permeability factors in experimental diabetes.* Br. J. Exp. Pathol. *57,* 747–754 (1976).

[7] B. M. Altura, S. Halevy and P. D. M. V. Turlapaty, *Vascular smooth muscle in diabetes and its influence on the reactivity of blood vessels.* Adv. Microcircul. *8,* 118–150 (1979).

[8] Z. B. Fortes, J. Garcia-Leme and R. Scivoletto, *Influence of diabetes on the reactivity of mesenteric microvessels to histamine, bradykinin and acetylcholine.* Br. J. Pharmacol. *78,* 39–48 (1983).

[9] Z. B. Fortes, J. Garcia-Leme and R. Scivoletto, *Vascular reactivity in diabetes mellitus: role of the endothelial cell.* Br. J. Pharmacol. *79,* 771–781 (1983).

[10] Z. B. Fortes, J. Garcia-Leme and R. Scivoletto, *Vascular reactivity in diabetes mellitus: possible role of insulin on the endothelial cell.* Br. J. Pharmacol. *83,* 635–643 (1984).

[11] A. G. Mowat and J. Baum, *Chemotaxis of polymorphonuclear leukocytes from patients with diabetes mellitus.* N. Engl. J. Med. *284,* 621–624 (1971).

[12] M. E. Miller and L. Baker, *Leukocyte functions in juvenile diabetes mellitus: humoral and cellular aspects.* J. Pediatr. *81,* 979–982 (1972).

[13] H. R. Hill, H. S. Sauls, J. L. Dettloff and P. G. Quie, *Impaired leukocyte responsiveness in patients with juvenile diabetes mellitus.* Clin. Immunol. Immunopathol. *2,* 395–403 (1974).

[14] A. E. Mohandes, J. L. Touraine, M. Osman and B. Salle, *Neutrophil chemotaxis in infants of diabetic mothers and in preterms at birth.* J. Clin. Lab. Immunol. *8,* 117–120 (1982).

[15] M. A. A. Pereira, P. Sannomiya and J. Garcia-Leme, *Inhibition of leukocyte chemotaxis by factor in alloxan-induced diabetic rat plasma.* Diabetes *36,* 1307–1314 (1987).

[16] S. Boyden, *The chemotactic effect of mixtures of antibody and antigen on polymorphonuclear leukocytes.* J. Exp. Med. *15,* 453–466 (1962).

[17] S. H. Zigmond and J. G. Hirsch, *Leukocyte locomotion and chemotaxis: new method for evaluation and demonstration of cell-derived chemotactic factor.* J. Exp. Med. *137,* 387–410 (1973).

[18] W. G. Spector, *The mediation of altered capillary permeability in acute inflammation.* J. Pathol. Bacteriol. *72,* 367–380 (1956).

[19] B. Desbuquois and G. D. Aurbach, *Use of polyethylene glycol to separate free and antibody-bound peptide hormones in radioimmunoassays.* J. Clin. Endocrinol. *33,* 732–738 (1971).

[20] P. Sannomiya, H. J. Anteghini, E. S. O. Vianna and J. Garcia-Leme, *Involvement of lymphocytes in non-immune inflammation:dual effect of glucocorticoids.* Agents and Actions *16,* 552–557 (1985).

[21] H. V. Showell, R. J. Freer, S. H. Zigmond, E. Schiffmann, S. Aswanikumar, B. Corcoran and E. L. Becker, *The structure-activity relations of synthetic peptides as chemotactic factors and inducers of lysosomal ensyme secretion for neutrophils.* J. Exp. Med. *143,* 1154–1169 (1976).

[22] E. J. Goetzl and W. C. Pickett, *Novel structural determinants of the human neutrophil chemotactic activity of leukotriene B.* J. Exp. Med. *153,* 482–487 (1981).

[23] C. L. Malmsten, J. Palmblad, A. M. Udén, O. Radmark, L. Engstedt and B. Samuelsson, *Leukotriene B4: a highly potent and stereospecific factor stimulating migration of polymorphonuclear leukocytes.* Acta Physiol. Scand. *110,* 449–451 (1980).

[24] M. J. H. Smith, A. W. Ford-Hutchinson and M. A. Bray, *Leukotriene B: a potential mediator of inflammation.* J. Pharm. Pharmacol. *32,* 517–518 (1980).

[25] H. S. Shin, R. Snyderman, E. Friedman, A. Mellors and M. M. Mayer, *A chemotactic and anaphylatoxic fragment cleaved from guinea pig C'5 by EAC' 1a, 4, 2a, 3.* Science *162,* 361–363 (1968).

[26] J. A. Jensen, R. Snyderman and S. E. Mergenhagen, *Chemotactic activity, a property of guinea pig C'5-anaphylatoxin.* In *Cellular and Humoral Mechanisms of Anaphylaxis and Allergy.* (Ed. H. Z. Movat) pp. 265–278, Karger, Basel 1969.

[27] P. A. Ward, *The heterogeneity of chemotactic factors for neutrophils generated from the complement system.* In *Cellular and Humoral Mechanisms in Anaphylaxis and Allergy.* (Ed. H. Z. Movat) pp. 279–288, Karger, Basel 1969.

[28] P. A. Ward and L. J. Newman, *A neutrophil chemotactic factor from human C'5.* J. Immunol. *102,* 93–99 (1969).

[29] L. Pillemer, M. D. Shoenberg, L. Blum and L. Wurz, *Properdin system and immunity. II. Interaction of the properdin system with polysaccharides.* Science *122,* 545–549 (1955).

[30] L. H. Muschel, K. Schmoker and P. M. Webb, *Anti-complementary actions of endotoxin.* Proc. Soc. Exp. Biol. Med. *117,* 639–643 (1964).

[31] O. Gotze and H. J. Muller-Eberhard, *The C3 activator system: an alternate pathway of complement activation.* J. Exp. Med. Suppl., vol *134,* 90–180 (1971).

[32] R. Snyderman and M. C. Pike, *Interaction of complex polysaccharides with the complement system: effect of calcium depletion on terminal component consumption.* Infect. Immun. *11,* 273–279 (1975).

[33] D. C. Morrison and L. F. Kline, *Activation of the classical and properdin pathways of complement by bacterial lipopolysaccharides (LPS).* J. Immunol. *118,* 362–368 (1977).

[34] R. Snyderman, H. S. Shin, J. K. Phillips, H. Gewurz and S. E. Mergenhagen, *A neutrophil chemotactic factor derived from C'5 upon interaction of guinea-pig serum with endotoxin.* J. Immunol. *103,* 413–422 (1969).

[35] S. Aswanikumar, B. Corcoran, E. Schiffmann, A. R. Day, R. J. Freer, H. J. Showell and E. L. Becker, *Demonstration of a receptor on rabbit neutrophils for chemotactic peptides.* Biochem. Biophys. Res. Commun. *74,* 810–822 (1977).

[36] L. T. Williams, R. Snyderman, M. C. Pike and R. J. Lefkowitz, *Specific receptor site for chemotactic peptides on human polymorphonuclear leukocytes.* Proc. Nat. Acad. Sci. USA *74,*1204–1208 (1977).

[37] S. J. Sullivan and S. H. Zigmond, *Chemotactic peptide modulation in polymorphonuclear leukocytes.* J. Cell Biol. *85,* 703–711 (1980).

[38] W. A. Marasco, J. C. Fantone, R. J. Freer and P. A. Ward, *Characterization of the rat neutrophil formyl peptide chemotaxis receptor.* Am J. Pathol. *111,* 273–281 (1983).

[39] D. W. Goldman and G. J. Goetzl, *Specific binding of leukotriene B4 to receptors of human polymorphonuclear leukocytes.* J. Immunol. *129,* 1600–1604 (1982).

[40] R. A. Kreisle and C. W. Parker, *Specific binding of leukotriene B4 to a receptor on human polymorphonuclear leukocytes.* J. Exp. Med. *157,* 628–641 (1983).

[41] D. E. Chenoweth and T. E. Hugli, *Human C5a and C5a analogs as probes of the neutrophil C5a receptor.* Mol. Immunol. *17,* 151–161 (1980).

[42] G. D. Warden, A. D. Mason, Jr. and B. A. Pruitt, Jr., *Suppression of leukocyte chemotaxis in vitro by chemotherapeutic agents used in the manegement of thermal injuries.* Ann. Surg. *181,* 363–369 (1975).

[43] J. B. Grogan, *Suppressed in vitro chemotaxis of burn neutrophils.* J. Trauma *16,* 985–988 (1976).

[44] S. M. Fikrig, S. C. Karl and L. Suntharalingam, *Neutrophil chemotaxis in patients with burns.* Ann. Surg. *186,* 746–748 (1977).

[45] J. M. Davis, P. Dineen and J. I. Gallin, *Neutrophil degranulation and abnormal chemotaxis after thermal injury.* J. Immunol. *124,* 1467–1471 (1980).