Allergen-induced Airway Inflammation in Rats
Role of Insulin

ELCIO O. VIANNA and J. GARCIA-LEME

Department of Pulmonology, School of Medicine, and the Department of Pharmacology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil

Clinical asthma appears to be less severe when diabetes mellitus is superimposed. To examine whether insulin influences the development of allergic reactions in the airway mucosa following antigen challenge, normal and diabetic rats sensitized against ovalbumin (OA) were used. Compared with controls, animals rendered diabetic by the injection of alloxan presented markedly decreased cell yields from bronchoalveolar lavage after OA challenge. The impaired response was not related to antibody production because enhanced IgE antibody titers of the same magnitude were found in both control and diabetic animals. Similarly, the mechanism underlying the inhibited responses could not be ascribed to hyperglycemia or intracellular glucopenia, first, because correction of blood glucose levels through fasting did not restore the decreased response, and second, because administration of 2-deoxyglucose, which blocks glucose utilization, did not affect the bronchoalveolar reaction to OA challenge in normal animals. Reversal of the impaired responses was attained by treatment of diabetic animals with insulin. There is evidence that insulin exerts proinflammatory effects. We conclude that insulin might modulate the inflammatory component of asthmatic responses. **Vianna EO, Garcia-Leme J. Allergen-induced airway inflammation in rats: role of insulin. Am J Respir Crit Care Med 1995;151:809–14.**

Both asthma and diabetes mellitus are common diseases, and concurrence is correspondingly expected to occur commonly. Several studies, however, indicate that the incidence of asthma in diabetic patients is less than its incidence in the residual population (1–4). In the same individual, clinical asthma appears to be less severe when diabetes is superimposed (2). In addition, the onset of the diabetic state is accompanied by diminution of symptoms of previously existing bronchial asthma(5).

The basic mechanism of asthma has long been thought to be bronchial obstruction, with bronchospasm and mucus hypersecretion playing a major role. However, there is a recent increase in the awareness of the importance of bronchial inflammation in asthma (6), with the recognition, over the past decade, that many features peculiar to asthma can be attributed to inflammatory processes (7). Experimental findings agree with the clinical evidence that disturbances of the inflammatory cycle are not uncommon in the diabetic state. Insulin receptors can be identified in the reacting structures of an inflamed area. Altered vascular responsiveness to inflammatory mediators, defective leukocyte-endothelial interactions, and inflammatory cell dysfunctions are described in insulin-deficient states. It is plausible, therefore, to assume that the activity of the reacting structures in inflammation is under the control of the ambient concentration of insulin (8, 9).

Direct evidence of inflammatory cells and their mediators in the airway mucosa and lumen after allergen challenge argues for an active role of cells in bringing about inflammatory changes (7, 10, 11). Bronchoalveolar lavage has been frequently used to document inflammatory cell involvement as a reflection of events at the surface of the airways and associated with late asthmatic response (12–15).

These observations prompted us to test the hypothesis that the continuing deficiency of insulin associated with diabetes mellitus influences the development of the inflammatory reaction in the airway mucosa following antigen challenge. Quantitative determinations of cells obtained by bronchoalveolar lavage and estimation of homocytrotopic antibody production in sensitized diabetic rats revealed an impaired response unrelated to antibody production that can be reversed by insulin treatment.

METHODS

Study Protocol

The experimental design included either sensitized or nonsensitized normal and diabetic male Wistar rats, 8 to 10 wk of age at the beginning of the experiments. The animals were either submitted or not to antigen provocation.

Sensitization to Ovalbumin and Antigen Provocation

Active sensitization against ovalbumin (OA) was performed by subcutaneous injection of 0.2 ml of a sterile solution containing 50 μg OA and 8 mg $Al(OH)_3$ in physiologic saline. Matching normal or diabetic control rats received an equal volume of physiologic saline containing the same amount of $Al(OH)_3$. The animals were anesthetized 14 d thereafter with an intraperitoneal injection of 300 mg/kg of chloral hydrate and the trachea exposed through a midline ventral incision of approximately 0.5 cm length in the neck. With the aid of a 26.5-gauge needle, 0.4 ml physiologic saline containing 2.5 mg/ml of OA was insufflated into the airway

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Correspondence and requests for reprints should be addressed to J. Garcia-Leme, M.D., Department of Pharmacology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, SP, Brazil 05508-900.

for antigen provocation. Controls received physiologic saline alone by the same route. Before closure of the incision, about 0.5 ml blood was collected from the jugular vein for assessment of antigen-specific antibody titers.

Bronchoalveolar Lavage

Bronchoalveolar lavage was performed 6, 16, or 24 h after OA challenge. The animals were anesthetized as referred to earlier, and the abdominal cavity was opened for blood collection and exsanguination from the abdominal aorta. The lungs were then lavaged by instillation of 5 ml physiologic saline, at room temperature, using a polyethylene tubing, 1 mm in diameter, inserted into the trachea. The procedure was repeated five times. The volume retrieved averaged 90% of the 25 ml that was instilled. Total cell counts were determined immediately using a hemacytometer (CC510;CELM, São Paulo, Brazil). Differential cell counts were carried out on stained blood films under oil immersion microscopy. A total of 200 cells was counted and classified as neutrophils, eosinophils, or mononuclear cells based on normal morphologic criteria.

Antibody Measurements

Antibody titers were estimated in duplicate by homologous passive cutaneous anaphylaxis (PCA), according to Watanabe and Ovary (16). Serum samples from venous blood collected at the moment of antigen provocation were injected intradermally into the shaven backs of normal recipients. The volume of intradermal injection was 0.1 ml on each site. After a latent period of 4 or 48 h, each animal was challenged intravenously with 0.5 ml of a solution containing OA and Evans blue dye in physiologic saline. The amounts of antigen and dye given were 1 and 25 mg/kg, respectively. The 4 h PCA was defined as IgG antibody titer and the 48 h PCA as IgE antibody titer. Evaluation of the PCA reactions was done estimating colorimetrically the content of exuded dye in the lesions. To do this, the animals were killed 30 min after the intravenous challenge, and the entire thickness of the skin reflected from the dorsal trunk. The content of dye in the lesions was estimated by excising the skin and holding the minced skin from each lesion in 10 ml formamide at 37° C for 24 to 30 h. The resultant solution was filtered and the optical density of the filtrate assessed at 650 nm. The concentration of dye in the filtrate was estimated from a standard graph recording the optical density of serial dilutions of a weighed sample of Evans blue in formamide (17, 18).

Induction of Diabetes Mellitus

Diabetes mellitus was induced by an intravenous injection of 40 mg/kg of alloxan dissolved in physiologic saline. Alloxan was injected 10 d before sensitization of the animals. The presence of diabetes was verified by blood glucose concentrations above 200 mg/dl determined with a blood glucose monitor in samples obtained from the cut tip of the tail immediately before antigen provocation. The presence of ketone bodies, as well as of glucose, was also qualitatively assessed in urine with the aid of reagent strips for urine analysis (Keto-Diastix®; Miles, São Paulo, Brazil).

Blood Analysis

Whole-blood samples, obtained from the abdominal aorta of the animals at the moment of bronchoalveolar lavage and collected in heparinized syringes, were used for assessing blood pH and blood gas tension (P_{CO_2} and P_{O_2}), oxygen saturation level, electrolyte concentration (sodium, potassium, chloride, ionized calcium, and bicarbonate), and hematocrit. Determinations were performed, immediately after blood collection, with the aid of an automatic, microcomputer-based system (Stat Profile ® analyzer; NOVA Biomedical, Waltham, MA). In a series of experiments, nonheparinized blood samples were employed for plasma osmolality determinations. Blood samples were centrifuged at 1,180 × g, for 25 min, and determinations made in 0.2 ml plasma aliquots using a wide-range osmometer (Advanced Instruments, Needham, MA). These measurements were made to evaluate potential abnormalities resulting from the induction of diabetes mellitus.

Blood Leukocyte Counts

Total and differential leukocyte counts were carried out in blood samples obtained from the cut tip of the tail of sensitized animals. Total counts were made in Neubauer chambers after samples were diluted 1:20 with Turk solution. Stained blood films were used for differential counts. Determinations were made immediately before challenge and 6 h thereafter.

Statistics

All results are expressed as mean ± standard error of the mean. The significance of differences between treatment groups was assess by Student's *t* test or analysis of variance with significant probability levels of less than 0.05.

Drugs and Chemicals

Alloxan monohydrate, ovalbumin (Grade III), and 2-deoxy-D-glucose were obtained from Sigma Chemical Co. (St. Louis, MO); $Al(OH)_3$ was from União Química (São Paulo, Brazil); Evans blue dye was from Merck (Darmstadt, Germany); and NPH insulin was supplied by Eli Lilly (São Paulo, Brazil).

RESULTS

Characteristics of Study Groups

The general characteristics of the animals in the different groups studied are shown in Tables 1 and 2. Blood pH, blood O_2 and CO_2 tension, oxygen saturation level, electrolyte concentrations, and hematocrit values remained within normal ranges in all groups. Ten animals in each group were randomly selected for blood analysis.

Animals rendered diabetic by the injection of alloxan exhibited a significant reduction in body weight gain during the experimental period relative to controls (35 ± 7 g, n = 66 and 89 ± 5 g, n = 104, respectively; $p < 0.05$). In addition, plasma glucose levels were sharply elevated in comparison with control levels (471 ± 24 mg/dl, n = 66 and 109 ± 25 mg/dl, n = 104, respectively; $p < 0.05$). Glycosuria was observed in this group, and ketone bodies were present in 20% of the animals.

Bronchoalveolar Lavage in Nondiabetic Animals

Compared with saline, OA challenge significantly increased the cell yield from bronchoalveolar lavage. Neutrophil and mononuclear leukocyte counts were elevated, maximal values being observed 6 h after challenge. A gradual return to basal levels, particularly in the case of mononuclears, was observed up to 24 h. Eosinophils were found in small numbers in lavage but did change significantly with OA challenge. In contrast, instillation of OA into the trachea of nonsensitized animals did not significantly increase cell yield in bronchoalveolar lavage (Table 3).

Diabetes Mellitus and Cell Yield in Bronchoalveolar Lavage Fluid

Animals rendered diabetic by the injection of alloxan 10 d before sensitization showed no significant increase in leukocyte counts in bronchoalveolar lavage fluid following OA challenge. Data obtained 6, 16, and 24 h after challenge in these animals showed a reduced cell yield throughout the experimental period. The finding suggests that diabetes mellitus did not merely alter the timing or kinetics of the cell response but rather interfered with the capacity of the animals to react normally to the antigen. Results are presented in Table 3.

Blood Leukocyte Counts in Sensitized Animals

Blood leukocyte counts performed immediately before and 6 h after OA challenge in nondiabetic and diabetic animals showed that under basal conditions total and differential counts were equivalent in both groups and that similar changes occurred during the 6 h interval following challenge. An increase of approximately 30% in total counts was observed, and this was caused

TABLE 1

BLOOD pH, Po_2 AND Pco_2, OXYGEN SATURATION LEVEL, AND BICARBONATE CONCENTRATION IN NORMAL AND DIABETIC RATS 6 H AFTER INTRATRACHEAL CHALLENGE*

Animals	Challenge	pH	Po_2 (*mm Hg*)	O_2 Saturation (%)	Pco_2 (*mm Hg*)	HCO_3^- (*mmol/L*)
Normal						
Nonsensitized	Saline	7.38 ± 0.01	86.7 ± 1.39	96.4 ± 0.24	41.5 ± 1.15	25 ± 0.65
Sensitized	OA	7.37 ± 0.01	88.5 ± 2.64	96.5 ± 0.32	40.7 ± 0.84	24 ± 0.31
Diabetic						
Nonsensitized	Saline	7.36 ± 0.02	89.4 ± 3.02	96.3 ± 0.41	42.2 ± 1.90	24 ± 1.43
Sensitized	OA	7.36 ± 0.03	86.7 ± 2.72	95.3 ± 0.32	36.8 ± 1.73	22 ± 0.76

* Blood samples were obtained from the abdominal aorta of 10 animals in each group. Diabetes mellitus was induced by the injection of alloxan 10 d before sensitization. Active sensitization against ovalbumin (OA) was performed 14 d before OA challenge.

TABLE 2

HEMATOCRIT VALUES AND BLOOD ELECTROLYTE CONCENTRATIONS IN NORMAL AND DIABETIC RATS 6 H AFTER INTRATRACHEAL CHALLENGE*

Animals	Challenge	Hematocrit (%)	Electrolyte Concentration (*mmol/L*)			
			Na^+	K^+	Cl^-	Ca^{2+}
Normal						
Nonsensitized	Saline	45 ± 0.69	140 ± 1.03	4.8 ± 0.18	105 ± 0.88	1.1 ± 0.02
Sensitized	OA	47 ± 0.75	138 ± 0.24	5.2 ± 0.05	105 ± 0.53	1.1 ± 0.03
Diabetic						
Nonsensitized	Saline	45 ± 0.89	137 ± 1.15	4.9 ± 0.16	104 ± 1.56	1.1 ± 0.01
Sensitized	OA	49 ± 0.71	136 ± 0.79	5.3 ± 0.19	102 ± 1.00	1.1 ± 0.04

* Blood samples were obtained from the abdominal aorta of 10 animals in each group. Diabetes mellitus was induced by the injection of alloxan 10 d before sensitization. Active sensitization against ovalbumin (OA) was performed 14 d before OA challenge.

TABLE 3

INFLAMMATORY CELLS IN BRONCHOALVEOLAR LAVAGE FLUID OBTAINED FROM NORMAL AND DIABETIC RATS AT VARYING INTERVALS AFTER INTRATRACHEAL CHALLENGE*

Animals	Challenge	Interval (*h*)	Cells × 10^6/Lavage			
			Total	Mononuclear	Neutrophils	Eosinophils
Normal						
Nonsensitized, n = 10	Saline	6	4.01 ± 0.29	3.97 ± 0.28	0.04 ± 0.03	0
Nonsensitized, n = 10	OA	6	4.63 ± 0.61	3.81 ± 0.59	0.82 ± 0.34	0
Sensitized, n = 8	Saline	6	3.70 ± 0.76	3.09 ± 0.69	0.61 ± 0.31	0
Sensitized, n = 11	OA	6	20.35 ± 1.59†	8.99 ± 1.91†	11.19 ± 1.75†	0.17 ± 0.07†
Sensitized, n = 6	OA	16	13.48 ± 4.38†	8.58 ± 3.33†	4.74 ± 1.20†	0.16 ± 0.10†
Sensitized, n = 10	OA	24	8.80 ± 1.09†	4.09 ± 0.75	3.96 ± 0.72†	0.75 ± 0.42†
Diabetic						
Nonsensitized, n = 10	Saline	6	4.73 ± 0.37	4.62 ± 0.39	0.11 ± 0.06	0
Nonsensitized, n = 6	OA	6	4.62 ± 1.07	3.98 ± 0.64	0.64 ± 0.45	0
Sensitized, n = 7	Saline	6	4.27 ± 0.61	4.26 ± 0.61	0.01 ± 0.01	0
Sensitized, n = 17	OA	6	6.70 ± 0.58‡	5.97 ± 0.63‡	0.70 ± 0.16‡	0.03 ± 0.01‡
Sensitized, n = 9	OA	16	6.04 ± 0.53‡	5.47 ± 0.40‡	0.58 ± 0.15‡	0‡
Sensitized, n = 7	OA	24	6.30 ± 0.62	5.36 ± 0.65	0.94 ± 0.20‡	0‡

* Animals were rendered diabetic by the injection of alloxan 10 d before sensitization. In both normal and diabetic rats, active sensitization against ovalbumin (OA) was performed 14 d before OA or saline intratracheal challenge.
† $p < 0.05$ compared with corresponding values in the first three groups of animals.
‡ $p < 0.05$ compared with corresponding values in normal animals.

by an increase in the number of granulocyte precursors and neutrophils. However, eosinophil counts were reduced, basophils were virtually absent from blood, and the number of mononuclear leukocytes remained unaltered in either group. Accordingly, the impaired cell yield from bronchoalveolar lavage observed in diabetic animals cannot be attributed to specific changes in peripheral blood leukograms (Table 4).

Antibody Measurements

PCA responses were used to estimate antibody production in normal and diabetic animals. Both control and diabetic rats presented increased IgE antibody titers of the same magnitude following sensitization with OA. Concentration-effect curves constructed from the response of test animals to passive antigen challenge showed a linear correlation between the magnitude of the reaction and the dilution of the serum, thereby indicating a proportionality between the amount of serum injected and the effect observed. Preheating of the samples at 56° C for 2 h completely destroyed the thermolabile IgE activity detected in samples. Negligible IgG antibody production was found in both groups of animals after OA sensitization. Results are shown in Figure 1.

TABLE 4

BLOOD LEUKOCYTE COUNTS IN NORMAL AND DIABETIC RATS BEFORE AND 6 H AFTER INTRATRACHEAL CHALLENGE*

	Cells/mm³							
Animals	Total	Myelocytes	Metamyelocytes	Stabs	Neutrophils	Eosinophils	Lymphocytes	Monocytes
Normal, n = 8								
Before	11,956 ± 1,136	0	22 ± 19	140 ± 62	3,332 ± 405	163 ± 56	8,046 ± 697	253 ± 58
After	16,075 ± 1,078†	16 ± 10†	118 ± 45†	822 ± 80†	7,074 ± 930†	31 ± 20†	7,802 ± 595	212 ± 47
Diabetic, n = 9								
Before	12,210 ± 1,606	0	25 ± 17	169 ± 81	3,062 ± 520	210 ± 62	8,460 ± 830	284 ± 53
After	15,736 ± 1,407†	18 ± 12†	131 ± 43†	857 ± 77†	6,520 ± 911†	47 ± 19†	7,907 ± 750	256 ± 61

* Animals were rendered diabetic by the injection of alloxan 10 d before sensitization. In both normal and diabetic rats, active sensitization against ovalbumin (OA) was performed 14 d before OA intratracheal challenge.

† $p < 0.05$ compared with corresponding values before challenge.

Cellular Glucopenia and Cell Yield in Bronchoalveolar Lavage Fluid

Nondiabetic, sensitized animals in which intracellular glucopenia, secondary to inhibition of glucose utilization, was induced by previous administration of 200 mg/kg of 2-deoxyglucose (2DG) exhibited a bronchoalveolar response to OA challenge that was indistinguishable from that of normal animals. 2DG dissolved in physiologic saline was injected by the intravenous route immediately before antigen provocation. Equivalent neutrophil, eosinophil, and mononuclear cell counts in the bronchoalveolar lavage of both groups of animals were found 6 h after OA challenge. Results are presented in Table 5.

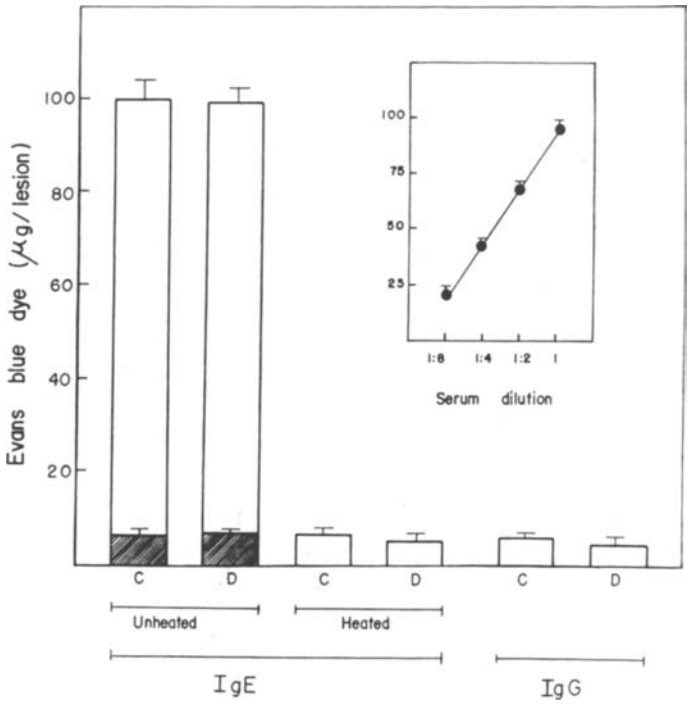

Figure 1. Antibody production in control (C, n = 84) and diabetic (D, n = 50) rats following active sensitization against ovalbumin (OA). Specific antibody titers were estimated by passive cutaneous anaphylaxis (PCA) 14 d after sensitization (see Methods). Hatched bars indicate IgE titers in nonsensitized animals. Preheating of serum samples at 56° C for 2 h completely destroyed the thermolabile IgE activity. IgG production was equivalent in sensitized and nonsensitized animals. (*Inset*) Concentration-effect curve showing a linear correlation between the intensity of the response and the amount of serum injected. Serum was obtained from nine control animals. For injection, serum samples were diluted (vol/vol) in Hanks' solution

Blood Sugar Levels, Plasma Osmolality, and Cell Yield in Bronchoalveolar Lavage Fluid

Normal and diabetic rats, previously sensitized against OA, were fasted for 15 h. Blood glucose levels and plasma osmolality were then determined and the animals submitted to OA challenge. Fasting reduced glycemic levels of diabetic animals to approximately normal values and slightly decreased such values in controls. Equivalent plasma osmolality values were found in both groups. Despite normal blood sugar and osmolality levels, the bronchoalveolar response of diabetic rats, determined 6 h after antigen provocation, was still reduced relative to that of controls. Results are presented in Table 6.

Influence of Insulin Treatment

Diabetic animals previously sensitized against OA were injected with 4 IU NPH insulin by the subcutaneous route immediately before OA challenge, and the bronchoalveolar lavage performed 6 h thereafter. Insulin treatment restored the capacity of the animals to respond to antigen provocation, as shown in Table 7.

DISCUSSION

The present study was designed to explore the mechanisms operative between the continuing deficiency of insulin, as observed in diabetes mellitus, and the anaphylactic response to ovalbumin injection into the airways of sensitized rats. Several animal species have been used successfully as models of human asthma and have yielded important clues to the mechanisms of the disease. These species include different rodents. The Wistar rat produces high IgE antibody titers to active immunization and develops airway inflammation after subsequent allergen challenge. This stresses the validity of the Wistar rat as a model for the study of lung allergic inflammation.

We found presently that, compared with controls, diabetic animals had a markedly reduced reaction to antigen challenge. This was demonstrated by a decreased cell yield from bronchoalveolar lavage. Determinations performed at various intervals following challenge showed that neutrophil, eosinophil, and mononuclear cell counts in diabetic rats were indistinguishable from those measured in nonsensitized animals, thereby indicating a complete loss of the capacity to react to antigen provocation.

The impaired responses accompanying the development of the diabetic state were unrelated to changes in peripheral blood leukograms, antibody production, blood glucose levels, or intracellular glucopenia. Control and diabetic rats had comparable blood leukocyte counts under basal conditions, and in both groups similar changes occurred following challenge. These include a considerable rise in the neutrophil count, which is characteristic

TABLE 5

INFLAMMATORY CELLS IN BRONCHOALVEOLAR LAVAGE FLUID OBTAINED FROM SENSITIZED RATS TREATED WITH 2-DEOXYGLUCOSE OR SALINE*

Treatment	Cells × 10^6/Lavage			
	Total	Mononuclear	Neutrophils	Eosinophils
Saline, n = 13	20.04 ± 3.59	9.46 ± 1.41	10.33 ± 2.41	0.25 ± 0.11
2-Deoxyglucose, n = 18	20.64 ± 2.02	10.86 ± 1.31	9.63 ± 1.27	0.15 ± 0.06

* Sensitization against ovalbumin (OA) was performed 14 d before OA challenge. Animals received either physiologic saline or 2-deoxyglucose (200 mg/kg), dissolved in physiologic saline, by the intravenous route immediately before challenge. Data were obtained 6 h thereafter.

TABLE 6

PLASMA OSMOLALITY, BLOOD GLUCOSE CONCENTRATION, AND INFLAMMATORY CELLS IN BRONCHOALVEOLAR LAVAGE FLUID OBTAINED FROM DIABETIC AND CONTROL RATS: EFFECT OF FASTING*

Animals	Plasma Osmolality (*mOsmol/L*)	Blood Glucose (*mg/dl*)	Cells × 10^6/Lavage			
			Total	Mononuclear	Neutrophils	Eosinophils
Control, n = 8	301 ± 2.95	79 ± 2.03	22.40 ± 2.11	7.19 ± 0.82	14.40 ± 1.91	0.81 ± 0.35
Diabetic, n = 7	302 ± 2.95	132 ± 10.05†	8.36 ± 1.09†	4.56 ± 0.47†	3.80 ± 1.20†	0

* Control (normal) and diabetic rats, sensitized against ovalbumin (OA) 14 d before, were fasted for 15 h. Blood glucose levels and plasma osmolality were then determined and the animals submitted to OA challenge. Data were obtained 6 h thereafter. Diabetes mellitus was induced by the injection of alloxan 10 d before sensitization.
† $p < 0.05$ compared with corresponding values in control group.

of the early stages of the inflammatory response and appears to reflect an enhanced mobilization of the cells from compartmental reserves (8), and eosinopenia at 6 h postchallenge, interpreted as a consequence of selective recruitment of these cells into the airways (7). Both control and diabetic rats exhibited enhanced IgE antibody titers of the same magnitude, whereas IgG antibody production was negligible in both groups. Rat anaphylactic antibodies are considered to be either of the reaginic type or associated with IgG-type globulin (19, 20). Because the immunization procedure used in the present study induced considerably higher IgE than IgG titers, this may imply that the anaphylactic response elicited in these animals was predominately mediated by reaginic-type antibodies. Despite equivalent IgE antibody production, however, diabetic animals were unable to react normally to the allergen. Similarly, the mechanism underlying the impaired responses cannot be ascribed to increased blood glucose levels or hyperosmolality secondary to hyperglycemia in diabetic animals because fasting rendered the animals normoglycemic and yet did not restore the response. Intracellular glucopenia is also unlikely to play a relevant role. Administration of 2-deoxyglucose prevents the penetration of glucose into cells (21), ultimately leading to inhibition of glucose utilization (22). Treatment of normal animals with 2-deoxyglucose did not influence the response to antigen provocation. The dose of 2-deoxyglucose used is sufficient to induce intracellular glucopenia (18). Accordingly, intracellular glucopenia that might occur in a circumstance of continuing deficiency of insulin does not appear to affect the capacity of sensitized animals to mount an immune response. In addition, blood pH, blood gas tension, oxygen saturation, plasma electrolyte concentrations, and hematocrit values, whose changes might be factors in variation of organic responses, remained within normal ranges in the group of diabetic animals (short-term diabetes mellitus).

TABLE 7

EFFECT OF INSULIN ADMINISTRATION ON INFLAMMATORY CELL YIELD FROM BRONCHOALVEOLAR LAVAGE OF DIABETIC ANIMALS*

Treatment	Cells × 10^6/Lavage			
	Total	Mononuclear	Neutrophils	Eosinophils
None, n = 10	5.63 ± 0.81	4.77 ± 0.78	0.81 ± 0.25	0.05 ± 0.05
Insulin, n = 9	18.11 ± 1.75†	7.72 ± 1.18†	10.11 ± 1.24†	0.28 ± 0.11

* Animals were rendered diabetic by the injection of alloxan 10 d before sensitization. Active sensitization against ovalbumin (OA) was performed 14 d before OA challenge. NPH insulin, 4 IU, was given by the subcutaneous route immediately before challenge. Data were obtained 6 h thereafter.
† $p < 0.05$ compared with corresponding values in untreated group.

Reversal of the impaired responses was attained by treatment of diabetic animals with insulin. A single dose of NPH insulin, given immediately before antigen challenge, was sufficient to produce a complete recovery of the animals, as indicated by bronchoalveolar lavages performed 6 h thereafter. The ability of insulin to restore altered responsiveness in experimental diabetes is an indication that the alterations may be primarily linked to continuing insulin deficiency. This suggestion is further supported by the observation that increased blood sugar levels, hyperosmolality secondary to hyperglycemia, or intracellular glucopenia were not directly responsible for the impaired responses occurring in diabetic animals. In general, the available data show that noticeable functional abnormalities are detected even at the early stages of diabetes mellitus and that in many instances they can be attributed to the relative lack of insulin. Quantitative alterations of inflammatory events in insulin-deficient states provide evidence supporting the proinflammatory effect of insulin. Decreased microvascular responses to inflammatory mediators (18, 23); reduced microvascular leakage and edema formation (24–26); deficient leukocyte-endothelial interactions and reduced accumulation of cells in inflammatory exudates (27, 28); granulocyte dysfunctions characterized by chemotactic, phagocytic, and intracellular killing deffects (29, 30); and impaired function of the mononuclear phagocytic system (9) are frequently associated with diabetes mellitus, leading to an enhanced susceptibility to infection in diabetic subjects.

Considerable attention is being focused on the role of inflammation in the pathophysiology of asthma. The underlying inflammatory reaction in this condition is viewed as a crucial component for the intensity of airways hyperresponsiveness, the chronicity of asthma, and often for the absence of complete therapeutic control when bronchodilator therapy is used alone (31,

32). Under normal conditions, the activity of the pancreatic islet B cells supplies optimal concentrations of circulating insulin, which is then immediately available to target structures. The vascular endothelium possesses surface binding sites for insulin (33). Furthermore, granulocytes, mononuclear leukocytes, lymphocytes, and cultured macrophages as well all have specific insulin binding sites with properties indistinguishable from those of insulin receptors characterized in mammalian tissues (34). The suggestion is therefore that insulin, acting as a proinflammatory hormone, is capable of controlling the inflammatory component of asthmatic responses.

The mechanisms underlying leukocyte accumulation in a tissue depend on the interaction between the cells and the vascular endothelium. Defective leukocyte-endothelial interactions are observed in diabetes mellitus. Under basal conditions, the number of cells in contact with the lining endothelium of postcapillary venules of diabetic rats is markedly reduced relative to controls. If a noxious stimulus is applied to induce a local lesion, leukocytes accumulate in the connective tissue of normal animals in a pattern characteristic of the inflammatory reaction, whereas in diabetic rats only a few cells are found in an equivalent area of the perivascular tissue (28). Several families of adhesion molecules that play a role in leukocyte-endothelial interactions have been identified. In a primate model of extrinsic asthma, a single inhalation exposure to an allergen induces the rapid (6 h) expression of a surface adhesive glycoprotein on vascular endothelium that correlates with the influx of neutrophils into the lungs and the onset of late-phase airway obstruction. Pretreatment with a monoclonal antibody to the glycoprotein blocks both the influx of neutrophils and the airway obstruction (35). Insulin may therefore play a regulatory role in the expression of cell adhesion molecules on the surface of the vascular endothelium and leukocytes. This would result in the impairment of inflammatory responses, including asthma, in insulin-deficient states.

Experimental evidence indicates that an overall reduced inflammation response is observed whenever a relative lack of insulin occurs in an organism (9). Interactions involving inflammatory and endothelial cells result in the production of inflammatory mediators. Therefore, in addition to reduced cell migration to the alveolar and airway lumen, impaired interstitial inflammation is likely to prevail in the diabetic state.

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References

1. Swern, N., N. J. Trenton. 1932. Incidence of diabetes mellitus in asthmatic patients. *J. Allergy* 2:375–378.
2. Abrahamson, E. M. 1941. Asthma, diabetes mellitus and hyperinsulinism. *J. Clin. Endocrinol.* 1:402–406.
3. Lasser, E. 1987. Asthma and diabetes mellitus: a biochemical basis for antithetical features. *Med Hypotheses* 23:95–106.
4. Casacó, A., D. Carbajal, and M. García. 1989. Bronchial asthma and diabetes mellitus. Experimental evidence of mutual exclusion. *Allergol Immunopathol* 17:105–108.
5. Helander, E. 1958. Asthma and diabetes. *Acta Med. Scand.* 162:165–174.
6. Bousquet, J., P. Chanez, A. M. Campbell, J. Y. Lacoste, R. Poston, I. Enander, P. Godard, and F. B. Michel. 1991. Inflammatory processes in asthma. *Int. Arch. Allergy Appl. Immunol.* 94:227–232.
7. Djukanovic, R., W. R. Roche, J. W. Wilson, C. R. W. Beasley, O. P. Twentyman, P. H. Howarth, and S. T. Holgate. 1990. Mucosal inflammation in asthma. *Am. Rev. Respir. Dis.* 142:434–457.
8. Garcia-Leme, J. 1989. Hormones and inflammation. Boca Raton, FL: CRC Press.
9. Garcia-Leme, J., and S. P. Farsky. 1993. Hormonal control of inflammatory responses. *Mediat. Inflamm.* 2:181–198.
10. Holgate, S. T., R. Djukanovic', J. Wilson, W. Roche, K. Britten, and P. H. Howarth. 1991. Allergic inflammation and its pharmacological modulation in asthma. *Int. Arch. Allergy Appl. Immunol.* 94:210–217.
11. Barnes, P. J. 1992. New aspects of asthma. *J. Intern. Med.* 231:453–461.
12. De Monchy, J. G. R., H. F. Kauffman, P. Venge, G. H. Koëter, H. M. Jansen, H. J. Sluiter, and K. De Vries. 1985. Bronchoalveolar eosinophilia during allergen-induced late asthmatic reactions. *Am. Rev. Respir. Dis.* 131:373–376.
13. Wardlaw, A. J., S. Dunette, G. J. Gleich, J. V. Collins, and A. B. Kay. 1988. Eosinophils and mast cells in bronchoalveolar lavage in subjects with mild asthma: relationship to bronchial hyperreactivity. *Am. Rev. Respir. Dis.* 137:62–69.
14. Diaz, P., M. C. Gonzalez, F. R. Galleguillos, P. Ancic, O. Cromwell, D. Shepherd, S. R. Durham, G. J. Gleich, and A. B. Kay. 1989. Leukocytes and mediators in bronchoalveolar lavage during allergen-induced late-phase asthmatic reactions. *Am. Rev. Respir. Dis.* 139:1383–1389.
15. Aalbers, R., H. F. Kauffman, B. Vrugt, M. Smith, G. H. Koëter, W. Timens, and J. G. R. De Monchy. 1993. Bronchial lavage and bronchoalveolar lavage in allergen-induced single early and dual asthmatic responders. *Am. Rev. Respir. Dis.* 147:76–81.
16. Watanabe, N., and Z. Ovary. 1977. Antigen and antibody detection by *in vivo* methods: a re-evaluation of passive cutaneous anaphylactic reactions. *J. Immunol. Methods* 14:381–390.
17. Wilhelm, D. L., P. J. Mill, E. M. Sparrow, M. E. Mackay, and A. A. Miles. 1958. Enzyme-like globulins from serum reproducing the vascular phenomena of inflammation. IV. Activable permeability factor and its inhibition in the serum of the rat and rabbit. *Br. J. Exp. Pathol.* 39:228–250.
18. Fortes, Z. B., J. Garcia-Leme, and R. Scivoletto. 1984. Vascular reactivity in diabetes mellitus: possible role of insulin on the endothelial cell. *Br. J. Pharmacol.* 83:635–643.
19. Ogilvie, B. M. 1967. Reagin-like antibodies in rats infected with the nematode parasite *Nippostrongylus brasiliensis*. *Immunology* 12:113–131.
20. Morse, H. C., K. F. Austen, and K. J. Bloch. 1969. Biological properties of rat antibodies. III. Histamine release mediated by two classes of antibodies. *J. Immunol.* 102:327–337.
21. Wick, A. N., D. R. Drury, H. I. Nakada, and J. K. Wolfe. 1957. Localization of the primary metabolic block produced by 2-deoxyglucose. *J. Biol. Chem.* 224:963–969.
22. Kipnis, D. M., and C. F. Cori. 1959. Studies of tissue permeability. V. The penetration and phosphorylation of 2-deoxyglucose in the rat diaphragm. *J. Biol. Chem.* 234:171–177.
23. Fortes, Z. B., J. Garcia-Leme, and R. Scivoletto. 1983. Vascular reactivity in diabetes mellitus: role of the endothelial cell. *Br. J. Pharmacol.* 79:771–781.
24. Llorach, M. A. S., G. M. Böhm, and J. Garcia-Leme. 1976. Decreased vascular reactions to permeability factors in experimental diabetes. *Br. J. Exp. Pathol.* 57:747–754.
25. Garcia-Leme, J., G. M. Böhm, R. H. Migliorini, and M. Z. A. Souza. 1974. Possible participation of insulin in the control of vascular permeability. *Eur. J. Pharmacol.* 29:298–306.
26. Gamse, R., and G. Jancsó. 1985. Reduced neurogenic inflammation in streptozotocin-diabetic rats due to microvascular changes but not to substance P depletion. *Eur. J. Pharmacol.* 118:175–180.
27. Perrillie, P. E., J. P. Nolan, and S. C. Finch. 1962. Studies on the resistance to infection in diabetes mellitus: local exudative cellular responses. *J. Lab. Clin. Med.* 59:1008–1015.
28. Fortes, Z. B., S. P. Farsky, M. A. Oliveira, and J. Garcia-Leme. 1991. Direct vital microscopic study of defective leukocyte-endothelial interaction in diabetes mellitus. *Diabetes* 40:1267–1273.
29. Pereira, M. A. A., P. Sannomiya, and J. Garcia-Leme. 1987. Inhibition of leukocyte chemotaxis by factor in alloxan-induced diabetic rat plasma. *Diabetes* 36:1307–1314.
30. Wertman, K. F., and M. R. Henney. 1962. The effects of alloxan diabetes on phagocytosis and susceptibility to infection. *J. Immunol.* 89:314–317.
31. Barnes, P. J. 1989. A new approach to the treatment of asthma. *N. Engl. J. Med.* 321:1517–1527.
32. Busse, W. W., W. F. Calhoun, and J. D. Sedgwick. 1993. Mechanism of airway inflammation in asthma. *Am. Rev. Respir. Dis.* 147:S20–S24.
33. Haskell, J. F., E. Meezan, and D. J. Pillion. 1985. Identification of the insulin receptor of cerebral microvessels. *Am. J. Physiol.* 248:E115–E125.
34. Bar, R. S., C. R. Kahn, and H. S. Koren. 1977. Insulin inhibition of antibody-dependent cytotoxicity and insulin receptors in macrophages. *Nature* (Lond.) 265:632–635.
35. Gundel, R. H., C. D. Wegner, C. A. Torcellini, C. C. Clarke, N. Haynes, R. Rothlein, C. W. Smith, and L. G. Letts. 1991. Endothelial leukocyte adhesion molecule-1 mediates antigen-induced acute airway inflammation and late-phase airway obstruction in monkeys. *J. Clin. Invest.* 88:1407–1411.