Leucocyte-migration Inhibition Induced by Uveoretinal Antigen in Patients with Diabetic Retinopathy

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SUMMARY

Leucocyte-migration inhibition was used to determine the state of hypersensitivity in 65 diabetic patients with different stages of retinopathy and 21 nondiabetic controls. About one third of the patients with simple or proliferative retinopathy exhibited significant leucocyte-migration inhibition to 0.2 mg./ml. protein concentration of uveoretinal antigen. In contrast, only one of 15 patients with minimal retinopathy and none of the controls showed significant leucocyte-migration inhibition. Corneal and lenticular antigens did not evoke a cellular immune response in any of the tested individuals. These findings suggest that cell-mediated hypersensitivity to uveoretinal antigen may develop in diabetic patients with prolonged, progressive, simple or proliferative retinopathy. DIABETES *25*:**1106-09, December, 1976.**

Leucocyte-migration inhibition (LMI) is used as an in-vitro test for cell-mediated hypersensitivity (CMH).[1] The test has demonstrated CMH to ocular tissue antigens in many ophthalmic disorders.[2] In a previous study, choroidal and retinal antigens induced significant (25 per cent or more) LMI in about one third of diabetic patients with proliferative retinopathy who were receiving photocoagulation therapy.[3] In the present report, diabetic patients with retinopathy of varying severity were examined by the LMI test using combined uveal-retinal antigen as well as other ocular antigens in an attempt to correlate the in-vitro cellular response with the stage of the diabetic retinopathy.

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MATERIAL AND METHODS

Patients. Sixty-five diabetic patients of the diabetic and eye clinics at Mount Sinai Hospital, New York, were divided into three groups according to the severity of their retinopathy (table 1). Group 1 consists of 15 diabetic patients who had minimal diabetic retinopathy—i.e., microaneurysms, punctate hemorrhages, and venous dilatation, but no exudates or neovascular formation. Almost all the patients in group 1 were adults with known diabetes of less than five years' duration, and one third of them were insulin-dependent. Group 2 was made up of 23 patients whose retinopathy included hard exudates, whereas group 3 comprises 27 patients with severe diabetic retinopathy evidenced by hemorrhages, exudates, and neovascular formation at the disc or at the retinal periphery. The patients from the last two groups were known to have clinical diabetes mellitus for at least 10 years, and 60 per cent of them were insulin-dependent (table 1). None had received photocoagulation therapy. Twenty-one nondiabetic subjects form the control group; they had normal retinae and were tested with the same antigen lots and at the same time as the diabetic patients.

Antigen was prepared, as previously described, from pooled human ocular tissues obtained from the New York Eye Bank.[3-5] In the present study, uveoretinal (UR), lenticular, and corneal-tissue-soluble extracts were used as antigens.

Buffy coat cells were obtained from the diabetic

TABLE 1

Clinical data

	No.	Duration (years)	Age of onset (years)		Age at testing (years)	Sex		Race		No. insulin-dependent
			< 25	> 35		F	M	W	N	
Diabetic patients with:										
minimal retinopathy	15	< 5	1	16	20-69	7	8	13	2	5 (33%)
simple retinopathy	23	> 10	4	19	38-82	13	10	14	11	14 (60%)
proliferative retinopathy	27	> 10	9	16	20-72	9	18	18	9	27 (100%)
Controls	21	—	—	—	22-65	13	8	15	6	—

patients and the healthy controls and tested for LMI, as previously described.[3-5] Approximately 12 capillary tubes packed with leucocytes could be obtained from each person. Sets of three capillary tubes were incubated in Sykes-Moore chambers with antigen or as controls without antigen. Each ocular antigen was tested in triplicate at 0.2 and 0.1 mg./ml. protein concentration. The results were expressed as percentage LMI, and 25 per cent LMI or more was considered significant migration inhibition.[3] A blood count was performed in all instances and a serum glucose level was determined in most of the diabetic patients when the LMI test was carried out.

RESULTS

LMI induced by 0.2 mg./ml. UR antigen in the three groups of diabetic patients and in the controls is depicted in figure 1. Approximately one third of the diabetic patients with simple or proliferative retinopathy responded with more than 25 per cent LMI, as opposed to one of 15 diabetic patients with minimal retinopathy and none of the 21 healthy controls ($p < 0.001$, χ^2 test). The percentage of individuals with significant inhibition (25 per cent or more LMI) was higher among the patients with proliferative retinopathy (11 of 27, or 40 per cent) than in the group with simple retinopathy (5 of 23, or 21 per cent), but the difference is not statistically significant ($p < 0.05$, χ^2 test). None of the diabetic patients or 15 normal subjects showed corneal or lenticular pathology or significant LMI by 0.2 mg./ml. corneal or lenticular protein. No correlation was found between the blood count, the serum glucose level, age of patient at time of testing, and LMI. No significant LMI was observed with 0.1 mg./ml. concentration of UR antigen in 15 assays.

To test the reproducibility of the results, nine diabetic patients and four healthy subjects were tested twice within a six-month period for LMI by UR antigen. Five of the seven patients with significant LMI in the first test had a similar response the second time. Controls and patients who showed no significant LMI did so on both occasions.

DISCUSSION

In the present study, significant LMI was induced by 0.2 mg./ml. protein concentration of pooled human uveoretinal (UR) antigen in diabetic patients with either simple or proliferative retinopathy. LMI was not observed with other ocular antigens. The in-vitro cellular response is similar to that observed with the same protein concentration from separate uveal tract and retinal antigens, although the combined UR antigen represents less protein from each tissue.[3] Moreover, the present study shows that cellular hyper-

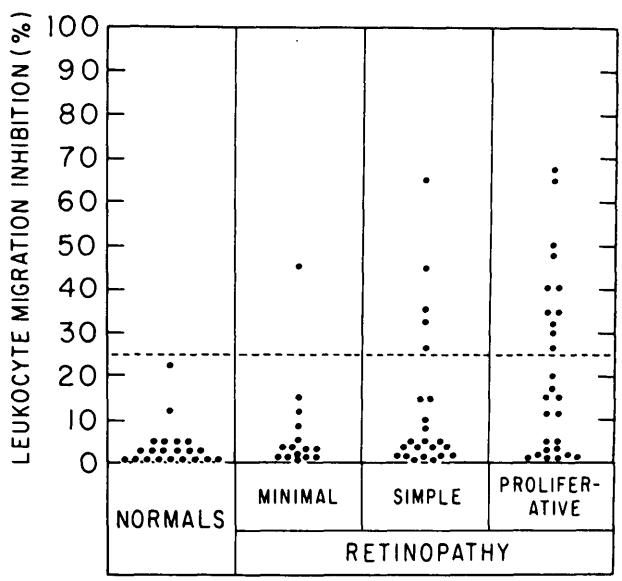

FIG. 1. Distribution of results of leucocyte-migration-inhibition test obtained in patients with diabetes mellitus who were grouped according to the severity of retinopathy and in nondiabetic controls. Uveoretinal-soluble proteins were used as antigen at a concentration of 0.2 mg./ml. Twenty-five per cent LMI is considered significant.

sensitivity to UR antigens is not confined to patients whose retinopathy has progressed to the proliferative stage[3] but can be demonstrated in patients with simple retinopathy. Since only one of 15 patients with minimal retinopathy exhibited LMI by UR antigen, as against a third of patients with advanced retinopathy, it appears that the development of immune response to uvea and retina is associated with exudative or proliferative retinopathy. The diabetic exudate, according to Michaelson et al.,[12] represents a cellular reaction mediated by monocytes and the reticuloendothelial system to the retinal damage caused by the diabetic process rather than a passive accumulation of fatty material. The finding of LMI by UR antigens in our patients may be related to the activity of mononuclear cells in the eye. However, the three groups of patients with diabetes mellitus who were compared in the present study differ not only in the degree of retinopathy but also in other parameters that may influence in an unknown way the development of CMH in diabetic patients. Thus, the group of patients with minimal retinopathy is noted to have a shorter duration and a later age of onset of the disease, as well as the lowest prevalence of insulin-dependent patients, than the other groups of diabetics (table 1). Indeed, leucocyte responsiveness has been reported to be impaired in patients with juvenile diabetes,[13] and phagocytosis was found to be reduced in polymorphonuclear leucocytes of poorly controlled nonketotic patients.[14,15] On the other hand, both LMI and lymphocyte transformation effectively demonstrated CMH to pancreatic antigens and to insulin in diabetic patients.[16,17]

The mechanism of the LMI test is influenced by the presence of both polymorphonuclear (PMN) and mononuclear cells.[6,7] When stimulated with the antigen, sensitized lymphocytes release a leucocyte-migration inhibition factor (LIF)[8] that prevents the migration of PMNs.[6] Antigen may also bind to cell-membrane antibody, thereby linking two or more antibody-bearing cells, causing cell aggregation, thus hindering migration.[9,10] Hence, the significance of the test as a correlate of CMH is still debatable.[6,11]

The reason for the appearance of cellular immune response to UR antigens in the diabetic patients is not known. The response, as evidenced by the LMI test, is not specific for diabetes and has been described in patients with prolonged chorioretinitis and retinal detachment.[18,19] Experimentally, immunopathogenic uveitis has been induced by intramuscular injections of rod outer segment and pigment epithelium.[20] It is possible that these tissues, which are relatively isolated from the circulation, may not be recognized as "self" by the surveying circulating lymphocytes. Exposure of circulating lymphocytes to these tissues as a result of the diabetic disease process may lead to autosensitization. However, the role of CMH to UR antigen in the formation of exudative or proliferative diabetic retinopathy and the role of various lymphokines, such as lymphotoxin and angiogenetic factor,[21] in its perpetuation and progression remains to be determined.

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