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Lymphocyte transformation test in sympathetic ophthalmitis and the Vogt-Koyanagi-Harada syndrome

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The lymphocyte transformation test (LTT) has been shown to be a reliable method of demonstrating the antigen of delayed-type allergic responses. The test is based upon the process of gradual enlargement, transformation into lymphoblasts, and mitotic activity of small mature lymphocytes in the peripheral blood which occurs when they come into contact with an antigen to which the organism has previously been sensitized (Pearman, Lycette, and Fitzgerald, 1963; Marshall and Roberts, 1963; Daniels, Ritzmann, and Levin, 1968; Ling, 1968). The mechanism of this blastic transformation appears to be identical with that of lymphocyte activation observed in the course of homograft rejection. Blastogenesis will not take place if no antigen has been added to the lymphocyte cultures, or if the donor organism has not been previously sensitized to the antigen tested.

Phytohaemagglutinin (PHA) is known to stimulate aspecifically the blastic transformation of lymphocytes cultured from healthy subjects (Hungerford, Donelly, Nowell, and Back, 1959; Nowell, 1960), but this stimulation does not occur if the subject suffers from a disease damaging the immune apparatus or is receiving immunosuppressive treatment (Daniels and others, 1968; Bozsóky, 1969).

In this paper the effects of uveal pigment and PHA on lymphocyte cultures from patients with sympathetic ophthalmitis (SO) or the Vogt-Koyanagi-Harada syndrome (VKHS) are compared.

Methods

LYMPHOCYTE CULTURES

These were taken from two patients suffering from the VKHS and three with SO. Cultures from five ophthalmologically healthy subjects served as controls. Diagnosis was based on the histories and typical clinical signs as well as the histological findings.

ANTIGENS

Uveal pigment not containing soluble proteins prepared from bovine eyes according to the method of Woods (1925) was used for specific stimulation. Phytohaemagglutinin M (0·01 U/ml.) manufactured by Difco was used for aspecific transformation.

CULTIVATION OF LYMPHOCYTES

Lymphocytes isolated from citrated venous blood by sedimentation were cultured in a 5-fold volume of Parker 199 solution (Simon, Dobozy, and Hunyadi, 1969). The lymphocytes were not separated

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from the other leucocytes during the experiments. The stimulating compounds were added at the start of cultivation, in 0·1 ml. volume per culture. At least three 3-ml. cultures were secured from each subject; the first was stimulated with appropriately diluted uveal pigment, the second with PHA, and the third, used as control, with isotonic saline.

After cultivation for 4 days, 3μ Ci H^3-thymidine was added in 0·1 ml. volume to each culture and cultivation was continued for an additional period of 24 hours. On the fifth day, 5 per cent. trichloracetic acid was added to the cultures to precipitate the macromolecules, and acid-soluble thymidine was removed by washing three times in trichloracetic acid. The scintillation counts of the DNA-containing sediment were established by means of a Tri-Carb apparatus (Packard).

The rate of stimulation was estimated from the increment of H^3-thymidine incorporation, a manifestation of the increased synthesis of DNA during blast transformation. The extent of blast transformation was assessed from the ratio of scintillation-counts in stimulated and non-stimulated cultures; the LTT was considered to be positive if this index was higher than 1·5.

Results

The concentration of uveal pigment eliciting the highest degree of blast transformation was determined first. Lymphocyte cultures from one patient with SO and from another with the VHKS were stimulated with different concentrations of the antigen. The incorporation of H^3-thymidine was highest at a final concentration of 2 μg/ml. uveal pigment. Higher and lower concentrations resulted in lower transformation indices (Table I). Uveal pigment was used at 2 μg/ml. level in all subsequent experiments.

Table I *Determination of optimal stimulating dose of uveal pigment*

Concentration of uveal pigment (μg./ml.)	*Quotient of scintillation counts (index) in cultures stimulated and not stimulated by uveal pigment*	
	VKHS	*SO*
200·0	1·4	1·2
20·0	1·9	2·1
2·0	2·3	2·6
0·2	2·0	1·8
0·02	1·3	1·4

Table II *Blast transformation stimulating effects of uveal pigment and PHA in lymphocyte cultures obtained from patients and healthy controls*

Patient no.	*Diagnosis*	*Uveal pigment*	*PHA*
1	VKHS	1·7	9·2
2		2·9	5·3
3	SO	2·1	6·7
4		1·6	8·8
5		2·4	5·0
6	Control	1·1	8·9
7		0·8	7·3
8		1·0	3·9
9		1·2	12·5
10		0·9	5·6

The figures represent quotients (indices) of scintillation counts in cultures stimulated and non-stimulated

The specific transformation elicited by uveal pigment yielded more than 1·5-fold increase of H^3-thymidine incorporation into the lymphocyte cultures of all patients suffering from SO or the VKHS. The same antigen failed to stimulate the lymphocyte cultures of the ophthalmologically healthy subjects. Blast transformation evoked by PHA was of much greater degree than that due to the specific antigen, but was of about the same magnitude in cultures from patients and healthy subjects (Table II).

Discussion

Elschnig (1910) and Woods and Little (1933) were the first to postulate the antigenic role of pigment in the pathogenesis of SO. This idea was supported by Friedenwald (1934), who compared the tissue-reaction after the intracutaneous administration of uveal pigment to the features of SO (Friedenwald, 1934); this is now interpreted as a delayed-type allergic response to the pigment. McPherson and Woods (1948) obtained identical results in the VKHS and SO with the uveal pigment skin-test. Kahán and Sztanojevits (1964) observed a correlation between the results of the skin-test and the activity of the above diseases. Uveal pigment agglutinating antibodies were demonstrated in the sera of patients suffering from the VKHS or SO if the combining antibodies were completed by Coombs-serum (Kahán, Sztanojevits, Szabados, Vass, and Szabó, 1964).

In the present experiments, the lymphocyte cultures of all patients with SO or the VKHS could be stimulated by uveal pigment, but the pigment consistently failed to enhance the incorporation of H^3-thymidine into cultures from healthy subjects.

These findings suggest that the uveal pigment has a specific transforming action upon the lymphocytes in these diseases. As most authors agree upon the reliability of the LTT in confirming delayed hypersensitivity, it is concluded that autoaggressive cell-bound responses to the pigment may play a role in the pathogenesis of both SO and the VKHS.

Summary

Lymphocyte cultures from two patients with histologically confirmed Vogt-Koyanagi-Harada syndrome and from three patients with sympathetic ophthalmitis were stimulated with bovine uveal pigment. The best antigen concentration was 2 µg/ml., which increased the blast transformation 1·6 to 2·9 times, as assessed by the incorporation of H^3-thymidine. The uveal pigment failed to stimulate the lymphocyte cultures from healthy subjects. The specific transforming action of uveal pigment indicates that these diseases are of a pigment autoaggressive delayed allergic nature.

References

BOZSÓKY, S. (1969) *Orvosképzés*, **44**, 49
DANIELS, J. C., RITZMANN, S. E., and LEVIN, W. C. (1968) *Texas Rep. Biol. Med.*, **26**, 5
ELSCHNIG, A. (1910) *v. Graefes Arch. Ophthal.*, **75**, 459
FRIEDENWALD, J. S. (1934) *Amer. J. Ophthal.*, **17**, 1008
HUNGERFORD, D. A., DONELLY, A. J., NOWELL, P. C., and BECK, S. (1959) *Amer. J. hum. Genet.*, **11**, no. 3, 215
KAHAN, Á., and SZANOJEVITS, A. (1964) *Szemészet*, **101**, 199
————, ————, SZABADOS, T., VASS, Z., and SZABÓ, M. (1964) *v. Graefes Arch. Ophthal.*, **167**, 246
LING, N. R. (1968) "Lymphocyte Stimulation". North-Holland, Amsterdam
MARSHALL, W. H., and ROBERTS, K. B. (1963) *Lancet*, **1**, 773
MCPHERSON, S. D., and WOODS, A. C. (1948) *Amer. J. Ophthal.*, **31**, 35
NOWELL, P. C. (1960) *Cancer Res.*, **20**, 462
PEARMAIN, G. LYCETTE, R. R., and FITZGERALD, P. H. (1963) *Lancet*, **1**, 637
SIMON, N., DOBOZY, A., and HUNYADI, J. (1969) *Arch. klin. exp. Derm.*, **236**, 1
WOODS, A. C. (1925) *Trans. ophthal. Soc. U.K.*, **45**, 208
———— and LITTLE, M. F. (1933) *Arch. Ophthal.*, **9**, 200