Effect of Kveim Antigen and Old Tuberculin on Lymphocytes in Culture from Sarcoid Patients

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In 1935 Williams and Nickerson used a saline suspension of sarcoid tissue intradermally in patients with sarcoidosis and obtained a positive delayed type of skin reaction. This test has been extended to include biopsy of the nodule produced by the injection at 28 days, and the observation in positive tests of a characteristic epithelioid cell reaction with giant cells. It is known in this form as the Kveim (1941) test. The Mantoux test is often negative in sarcoidosis, yet by passive transfer of lymphocytes from a Mantoux-positive subject a positive reaction may be obtained (Urbach *et al.*, 1952).

D'Arcy Hart *et al.* (1964), in a detailed study on the association between the Kveim test, previous B.C.G. vaccination, and tuberculin sensitivity, have shown that in a group of clinically normal subjects positive Kveim tests were rather more frequent and specific nodules much larger in those who did not show conversion to tuberculin sensitivity after two B.C.G. vaccinations. The most likely explanation for this phenomenon was that the vaccination was responsible for some immunological change which manifested itself in a positive Kveim test and was more prominent in subjects with persistent tuberculin anergy.

In patients with sarcoidosis, B.C.G. vaccination fails to achieve sustained Mantoux conversion (Forgacs *et al.*, 1957), although evidence of latent tuberculin sensitivity may be found in sarcoidosis using tuberculin in oily emulsion (James and Pepys, 1956).

Thus it appears that while certain delayed hypersensitivity reactions in sarcoidosis are diminished (*Brit. med. J.*, 1958), skin reactivity can be induced by passive transfer of lymphocytes.

The defect in the tuberculin reaction in sarcoidosis seems, therefore, to be at a cellular level, since leucocytes from tuberculin-insensitive patients with sarcoidosis have been shown by means of tissue-culture techniques to be deficient in tuberculin antibody (Citron, 1958). We set out to test the reaction of lymphocytes in culture from proved cases of sarcoid to old tuberculin and to Kveim reagent. Permain *et al.* (1963) and Elves *et al.* (1963) have shown that in normal subjects sensitized

to a bacterial antigen this antigen will cause a proportion of their lymphocytes to undergo blastic transformation in culture.

Cultures were set up as previously reported (Quaglino *et al.*, 1962). Old tuberculin (O.T.) was used in a final dilution of 1:25,000 and in some 1:250, these dilutions having been shown previously (Cowling *et al.*, 1963) to produce a satisfactory response in Mantoux-positive subjects. To some cultures hydrocortisone succinate in a dilution of 2 mg./100 ml. was added. This was done because Pyke and Scadding (1952) had found that cortisone might cause patients with sarcoidosis to show tuberculin sensitivity.

Kveim reagent prepared by Dr. L. E. Siltzbach and kindly supplied by Dr. D. Geraint James was used at a final dilution of 1:50. The reagent is particulate, but this was thought unlikely to affect its activity in culture, since blastic transformation has been observed previously in experiments using the particular T.A.B. antigens in culture. Seven patients and two normal Mantoux-positive controls were studied. The results are set out in the Table. The figures given are the percentage of lymphoid blast cells after three days of culture. After five days similar findings were observed.

These findings indicate a failure of circulating lymphocytes to react either to O.T. or to Kveim reagent in patients with sarcoidosis, although they showed normal growth with phytohaemagglutinin M (P.H.A.), the most recent cases showing the least response. It seems likely that in this condition the production of lymphocytes reactive to tuberculin and presumably capable of producing tissue-sensitizing antibody is impaired. This further supports the view that the altered tissue reactivity in sarcoidosis is due to a failure of circulating lymphocytes to transfer hypersensitivity to tissue.

The failure of Kveim reagent to produce transformation of lymphocytes in patients with sarcoidosis is of interest and suggests that the very much delayed type of skin reaction produced by this reagent, if it is indeed an example of delayed hypersensitivity, may not be mediated by lymphocytes.

SUMMARY

Leucocyte cultures were set up in Mantoux-positive normal subjects and patients with sarcoidosis. Old tuberculin and Kveim reagent were added to see whether these reagents would induce transformation of lymphocytes.

O.T. produced no transformation of lymphocytes in seven subjects with sarcoidosis and Kveim reagent no transformation in three when the cultures were observed for five days.

Results in Two Controls and Seven Patients

Subject	Mantoux Test	Biopsy	Clinical Type	Duration of Disease (Years)	Current Steroid Therapy	O.T. 1:25,000	O.T. 1:250	O.T. 1:250 + Hydrocortisone Succinate (2 mg./100)	Kveim Reagent 1:50	P.H.A. 1:25
C1	Positive		Normal control		No	5%	n.e.	n.e.	0%	71%
C2	"		"		"	8%	3%	"	0%	72%
P1	Negative	n.e.	Erythema nodosum. Pulmonary infiltration. Hilar enlargement	1½	Yes	0%	0%	0%	n.e.	64%
P2	"	Liver +	Pulmonary infiltration	1	No	0%	0%	0%	"	54%
P3	"	"	Pulmonary infiltration. Hilar enlargement	2½	Yes	0%	0%	0%	"	69%
P4	"	Scalene node +	Hilar enlargement	2½	No	0%	0%	0%	"	75%
P5	"	Liver +	Pulmonary infiltration. Hilar enlargement	9	"	0%	n.e.	n.e.	0%	72%
P6	"	n.e.	Erythema nodosum. Hilar enlargement	3	"	0%	"	"	0%	73%
P7	"	"	Erythema nodosum. Pulmonary infiltration. Hilar enlargement	½	Yes	0%	"	"	0%	46%

The percentages shown are those of lymphoid blast cells (lymphoblasts and prolymphocytes) after three days of culture. n.e. = Not examined.

This supports the view that anergy to tuberculin in sarcoidosis is due to an absence of lymphocytes in the circulation capable of reacting to tuberculin, and also suggests that the Kveim reaction is, unlike the classical delayed hypersensitivity reaction, probably not mediated by lymphocytes.

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Part Played by Calcium in Sympathetic Stimulation

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Evidence has been published recently that calcium is a key substance for the release of the catechol amines, adrenaline and noradrenaline, from the adrenal medulla. Splanchnic nerves transmit their impulses to the adrenal medulla by releasing acetylcholine. Douglas and Rubin (1961, 1963) perfused the adrenal gland with Locke's solution, stimulated the medulla by injecting acetylcholine into the artery leading to the gland, and measured the catechol amines liberated in the adrenal vein. They found that the amount liberated depended on the amount of calcium in Locke's solution. They showed that calcium was the only cation necessary for the release, since acetylcholine was effective when the perfusing fluid contained nothing more than sucrose, dextrose, and calcium. They suggested that acetylcholine either depolarized the membrane of the adrenal medullary cells or altered its permeability, so that calcium was able to enter the cells and to release catechol amines from the granules inside them. The amount of calcium entering depended on the external concentration of calcium, and therefore the amount of catechol amines released also depended on the external concentration of calcium.

SYMPATHETIC POST-GANGLIONIC FIBRES

Burn and Rand (1959) have put forward the hypothesis that the impulse passing down the sympathetic post-ganglionic fibre releases acetylcholine and that this in turn releases noradrenaline. The question thus arose how far calcium played a similar part in the release of noradrenaline from the sympathetic fibre to that which it played in the release of catechol amines from the adrenal medulla.

In order to make observations the preparation of the rabbit ileum described by Finkleman (1930) was used, in which a loop of ileum is suspended in an isolated organ bath and stimulation is applied to the periaxillary nerves in the adjacent mesentery. This stimulation causes inhibition of the pendular movements of the ileum.

A known number of supramaximal shocks were given to the periaxillary nerves, usually at a frequency of 10/sec., and the inhibition was observed in Locke's solution or Tyrode's solution containing the normal amount of calcium, approximately 2 mM. The calcium concentration was then increased in steps, twofold, fourfold, and eightfold. The inhibition was very much greater in twice normal calcium, again very much greater in

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four times normal calcium, and more prolonged in eight times normal calcium. On reducing the calcium to normal, stimulation produced an inhibition similar to that seen at first. Thus the effect of sympathetic stimulation was increased as the calcium concentration was raised.

EXPERIMENTAL RESULTS

Effect of Calcium on Response to Noradrenaline.—The increased inhibition could be explained in two ways. It could be due to an increase in the amount of noradrenaline released, or it could be due to an increase in the response to the same amount of noradrenaline. We were able to test this by comparing the inhibition caused by stimulation with the inhibition caused by adding noradrenaline to the bath. In raising the calcium from one-third normal to normal there was a great increase in the inhibition produced by stimulation in each of seven experiments, but there was an increase in the inhibition produced by noradrenaline in only one. On the other hand, in raising calcium from normal to twice normal there was some increase in the inhibition produced by noradrenaline in six out of seven experiments. This increase, however, did not appear to be as great as the increase in the response to stimulation. The results showed that the increase in the response to stimulation as the calcium concentration was increased was mainly due, particularly in the lower ranges, to an increase in the amount of noradrenaline released.

Raising Calcium During Continuous Stimulation.—Perhaps the most significant results were obtained by raising the calcium concentration during a period of continuous stimulation. An example of this is shown in the Chart, in which stimulation at a frequency of 10/sec. was applied in a solution containing

Contractions of isolated rabbit ileum in Tyrode's solution containing 0.6 mM. calcium. Periaxillary nerves in the mesentery stimulated at 10/sec. during the horizontal line. The contractions were inhibited to about 50%. At D.S. the drum was stopped for 5 min. At the arrow the calcium concentration was raised to 1.8 mM., and the inhibition was greatly increased.