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THE RELATIONSHIP BETWEEN LYMPHOCYTE TRANSFORMATION AND IMMUNE RESPONSES

II. CORRELATIONS BETWEEN TRANSFORMATION AND HUMORAL AND CELLULAR IMMUNE RESPONSES

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SUMMARY

Rabbits were immunized against purified proteins, tissue extracts or sheep erythrocytes, with or without Freund's adjuvant. Skin reactions of the delayed type were found only in animals given antigen with adjuvant, although all rabbits developed serum antibodies and most of them Arthus type reactions. Lymphocytes of all animals, however, showed similar transformation activity when cultured with the immunizing antigen.

Thus the blast transformation phenomenon correlates well with both cellular and humoral immune responses and not with the delayed type hypersensitivity only.

INTRODUCTION

The blast transformation reaction is becoming a useful tool in clinical and experimental immunology. This reaction may be a useful test for hypersensitivity of patients to different antigens, as reported by Girard *et al.* (1967) and Halpern *et al.* (1967). Other clinical applications may be the mixed lymphocyte reaction for tissue typing (see Wilson & Billingham, 1967).

Mills (1966), Zweiman (1967) and Oppenheim (1968) claimed a close correlation, in immunized animals, between blast transformation and the cellular, but not humoral, immune response. Others, however (Benezra, Gery & Davies, 1967b; Loewi, Temple & Vischer, 1968) showed that specific lymphocyte transformation activity may be detected in the blood of immunized animals with no delayed hypersensitivity. Similar conclusions were drawn also by Girard *et al.* (1967), Halpern *et al.* (1967) and Fellner *et al.* (1967), who tested the reactions of allergic patients. Most of these patients exhibited allergy of the immediate type only, but the lymphocyte responses were similar whether delayed hypersensitivity existed or not.

We report here in detail our findings with rabbit lymphocytes, which give further support

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to the assumption that blast transformation is in correlation with both cellular and humoral immune responses.

MATERIALS AND METHODS

Animals

Adult mongrel rabbits of both sexes, weighing 2·5–4 kg were employed.

Antigens

Bovine, rat, chicken and rabbit heart and bovine and rat kidney homogenates were prepared as described by Gery & Davies (1961). The extract supernatants for immunization were obtained by centrifugation at 12,000 *g* for 30 min; for stimulation of lymphocyte cultures and skin tests, the 12,000-*g* supernates were centrifuged in sterile conditions at 25,000 *g* for 60 min.

Sheep red blood cells (SRBC) were stored in sodium citrate and washed three times in saline before use. Bovine serum albumin (BSA), crystallized and bovine serum γ-globulin (BGG) were purchased from Armour Pharmaceutical Co., Kankakee, Illinois.

Immunization

The tissue extracts were injected in saline or emulsified (1 : 1) in complete Freund's adjuvant (Difco) enriched with 5 mg/ml tubercle bacilli (human type, strain C, kindly supplied by the Ministry of Agriculture, Fisheries and Food, Central Veterinary Laboratory, Weybridge, Surrey, England). One millilitre of the saline tissue extracts, containing about 20 mg protein was injected intraperitoneally and subcutaneously to each rabbit. The tissue extracts with adjuvant were injected into the hind footpads and subcutaneously, each rabbit receiving 1 ml of the emulsion, containing about 15 mg protein.

BSA was injected in saline solution, in enriched Freund's adjuvant, or as alum precipitate. The alum precipitate was prepared by mixing BSA solution (10 ml of 2%) with $NaHCO_3$ (0·27 ml of 10%) and $KAl(SO_4)_2$ (2·5 ml of 4%). The precipitate was incubated overnight in the cold before being used. One millilitre of precipitate (about 15 mg protein) was injected into each rabbit by the subcutaneous route. The saline solution and emulsified antigen were injected in quantities and routes similar to those described above for the tissue extracts.

Two rabbits received 20 mg of BGG, without adjuvant, subcutaneously and intraperitoneally, after heat aggregation at 60°C for 30 min with shaking.

SRBC were given intravenously, 1·0 ml of 15% suspension being injected into the marginal vein of the ear of each rabbit.

The booster immunization given to animals consisted of 10 mg antigen protein in saline, inoculated subcutaneously.

Lymphocyte cultures

Blood samples were drawn by heart puncture 3–6 weeks after immunization. The cultures, set as described by Benezra, Gery & Davies (1967a), were stimulated by 100 μg of the immunizing proteins or tissue antigens or by 0·1 ml of 2% SRBC. The degree of response was determined after 5 days' incubation by both percentage of transformed lymphocytes

and uptake of tritiated thymidine (^{3}HT) (Benezra *et al.*, 1967a; Gery, Benezra & Davies, 1969).

Skin reactions

These tests were carried out by intradermal injection of 100 μg of the 25,000-*g* tissue extracts, 0·1 ml of 0·1% SRBC suspension or 30 μg of the purified proteins. The results were read after 4 hr for the Arthus reaction and after 24 and 48 hr for the delayed type hypersensitivity. The reactions were recorded as diameter of induration (mm), with the haemorrhage and necrosis being evaluated by an arbitrary scale of 1–3.

Detection of serum antibodies

The capillary precipitation and passive haemagglutination methods (Gery & Davies, 1961) were used for assessing the titres of antibodies against proteins or tissue extracts.

RESULTS

Tissue extracts as antigens

Rabbits immunized against heterologous (xenogeneic) organ extracts in enriched Freund's adjuvant showed both Arthus reactions of 10–16 mm with haemorrhage and intense delayed type hypersensitivity with 11–22 mm reactions and necrosis (Table 1). The lymphocytes collected from these rabbits showed marked blast transformation responses when stimulated specifically *in vitro*, with 8·9–41·1% transformed cells and increased metabolic activity, demonstrated by ^{3}HT uptake ratio of 4·5–113·4 between experimental and control cultures.

TABLE 1. Correlation between lymphocyte transformation and humoral and cellular immune reactions in rabbits immunized with tissue extracts and Freund's adjuvant

Rabbit	Immunizing antigen	Skin test*				Serum antibodies		Blast transformation	
		Arthus		Delayed					
		Diameter (mm)	Haemorrhage	Diameter (mm)	Necrosis	Haemagglutination titres	Precipitins	Blasts† (%)	Thymidine uptake‡
P10	Rat heart	14	++	20	+	—	+++	14·3	68·2
P11	Rat heart	10	±	16	++	>31,250	+++	9·9	14·2
P12	Rat heart	16	+++	22	+++	31,250	+++	11·8	32·7
P15	Rat kidney	16	++	20	+	1,250	+++	12·0	113·4
P16	Rat kidney	14	++	15	+	—	+	8·9	4·5
P13	Beef heart	12	+	11	+	1,250	+++	22·3	48·2
P14	Beef heart	12	+++	22	+++	—	+++	41·1	47·9
P20	Rabbit heart	0	0	0	0	0	0	0	1·5

* Reactions below 8 mm were considered negative.
† Net percentage after subtraction of control percentage.
‡ ^{3}HT counts with antigen/^{3}HT counts in control culture.

Rabbits immunized against heterologous tissue extracts without adjuvant (Table 2) showed Arthus reactions of 11–17 mm with haemorrhage, but none of these animals exhibited significant skin reactions of the delayed type. All rabbits of this group gave marked transformation reactions, with 2·8–28·1% blast cells and ^{3}HT uptake ratios of 6·3–134·0. It is of interest that the animals given booster injections showed higher levels of transformation (Table 2).

Serum antibodies, shown by capillary precipitation and passive haemagglutination, were present in rabbits immunized by extracts in either saline or adjuvant. There was no correlation between the titres and the level of lymphocyte transformation.

Rabbit P20 (Table 1) and other animals immunized against homologous (allogeneic) heart extract, not included in the tables, did not respond by any test to the immunizing antigen.

TABLE 2. Correlation between lymphocyte transformation and humoral and cellular immune reactions in rabbits immunized without adjuvant

Rabbit	Immunizing antigen	Skin test				Serum antibodies		Blast transformation	
		Arthus		Delayed					
		Diameter (mm)	Haemorrhage	Diameter (mm)	Necrosis	Haemagglutination titres	Precipitins	Blasts (%)	Thymidine uptake
P41	Rat heart	15	++	0	0	1,250	+	10·2	13·1
P46	Rat kidney	17	++	0	0	250	++	6·4	18·1
P43	Beef heart	15	++	0	0	1,250	++	23·0	78·0
P44*	Beef heart	12	++	0	0	—	+++	25·9	134·0
P45	Beef heart	13	++	0	0	—	++	20·3	6·3
P47	Beef kidney	15	++	0	0	6,250	+++	4·9	8·6
P48	Beef kidney	11	+	0	0	1,250	++	2·8	9·8
C7*	Chicken heart	17	++	0	0	—	++	20·7	59·4
C8*	Chicken heart	15	++	0	0	—	++	28·1	73·0

See legend of Table 1 for further explanations.

* Tested after booster injection.

Soluble proteins as antigens

Table 3 summarizes the reactions of rabbits immunized against BSA in adjuvant emulsion or alum precipitate and against heat aggregated BGG. Delayed hypersensitivity was found only in rabbits immunized with the addition of adjuvant, and these showed reactions of 9–25 mm with necrosis. Humoral antibody response and Arthus type of skin reactions, on the other hand, were provoked by all immunization procedures, although the reactions were more intense in rabbits inoculated with adjuvant. Positive transformation reactions too were found in all immunized rabbits, with higher levels being shown on the whole by cells from those immunized with adjuvant. Individual variations affected the intensity of all reactions, as shown by rabbit A13 with low responses in all tests.

TABLE 3. Correlation between lymphocyte transformation and humoral and cellular immune reactions in rabbits immunized without any adjuvant, with alum or with Freund's adjuvant

Rabbit	Immunizing antigen	Skin test				Serum antibodies		Blast transformation	
		Arthus		Delayed					
		Diameter (mm)	Haemorrhage	Diameter (mm)	Necrosis	Haemagglutination titres	Precipitins	Blasts (%)	Thymidine uptake
A5	BSA+alum	12	+	0	0	1,250	++	10·0	8·5
A6	BSA+alum	10	++	0	0	—	++	27·9	11·7
A11	BSA+adjuvant	20	++	22	+++	6,250	+++	20·7	68·1
A12	BSA+adjuvant	16	++	19	+++	31,250	+++	21·7	37·5
A13	BSA+adjuvant	10	+	9	±	250	+	3·1	2·2
A14	BSA+adjuvant	18	+++	25	+++	>31,250	+++	40·0	10·8
G1	BGG aggregated	8	±	0	0	1,250	+	4·0	4·7
G2	BGG aggregated	9	+	0	0	6,250	+	7·1	5·3

See legend of Table 1 for explanations.

Red blood cells as antigens

More than thirty rabbits were immunized intravenously with sheep erythrocytes. Lymphocyte cultures from most of these animals showed marked transformation activity when stimulated by the specific immunizing antigen (Gery *et al.*, 1969; Gery, Eyal & Benezra, 1969). These animals formed serum antibodies but none of those skin tested showed either Arthus or delayed response.

DISCUSSION

The data reported here show that a correlation exists in general between antigen-induced blast transformation and other immunological manifestations. Rabbits with strong immune responses usually showed more intense transformation reactions than those with weak immunity against the same antigens. Strong immune responses were induced in the present experiments by use of adjuvant emulsion, or by repeating injections of antigen in saline (boosters). The rabbits immunized against antigen with adjuvant were the only ones which developed the delayed type of hypersensitivity but our studies do not support the assumption that this type of immunity is the only one which is in direct correlation with the blast transformation response. Rabbits challenged with single or repeated injections of different antigens in saline did not show detectable delayed hypersensitivity. All these animals, however, demonstrated considerable degrees of transformation activity, as well as humoral antibodies. The results presented here are in line with those of Loewi *et al.* (1968) and with the data concerning the blast transformation reaction of allergic patients (Girard *et al.*, 1967; Halpern *et al.*, 1967; Fellner *et al.*, 1967). Most of these patients did not carry the delayed type of hypersensitivity but showed activity of humoral antibodies (reagins), with positive transformation reactions. Furthermore, Fellner *et al.* (1967) reported that the lymphocytes from two of their patients were not stimulated by the specific antigen in culture,

in spite of the presence of delayed skin reactivity. These findings are in contradiction with the conclusions of Mills (1966), Zweiman (1967) and Oppenheim (1968), that the transformation reaction is directly correlated with the cellular (delayed type) immune response. The data of these authors were obtained from experimental animals and some antigenic systems consisted of hapten–protein conjugates. The lymphocyte reactions in these systems showed specificity toward the conjugates rather than the hapten, similar to the specificity of the delayed hypersensitivity. This specificity of lymphocyte reaction can be interpreted, however, to be due to the ability of sensitized cells to react with large molecules only and, therefore, the immunizing protein cannot be substituted by another carrier for this cellular reaction. This assumption is in accordance with the results of Paul, Siskind & Benacerraf (1968), who could show minute stimulation of lymphocytes by conjugates with non-immunizing carriers, whereas the reactions stimulated by the immunizing conjugate were more intense by far. Mills could not show transformation activity in lymph node cells from animals immunized intravenously and later into the footpads. This author did not examine the spleen lymphocytes of these animals and in view of the studies of Vischer & Stastny (1967), it may be suggested that the transformation activity was mainly localized in this organ.

Further support of the hypothesis that lymphocyte transformation relates to delayed hypersensitivity has been adduced by Bloom & Bennett (1968) who found that sensitized lymphocytes cultured with specific antigen, release into the medium the migration inhibitory factor (MIF), which is specific for delayed hypersensitivity. These cells were found to release also some factor(s) that stimulate normal lymphocytes to transform *in vitro*. These authors suggest that MIF is identical with the transformation stimulating factor(s) but the presence of blast transformation in animals without delayed hypersensitivity puts this suggestion in doubt.

The mentioned data may be explained by one of two hypotheses:

(1) The lymphocytes responsible for the transformation response may also bear the capacity to participate in reactions of delayed type hypersensitivity.

(2) The two cellular functions are carried out by two different cell populations; the transforming lymphocytes being produced in animals with both cellular or humoral immunity, whereas cells of the other type are formed only in animals with delayed hypersensitivity.

The presented results do not eliminate either of these hypotheses, although other studies have shown that the population of cells producing humoral antibodies is not identical with that of transforming lymphocytes (Gery *et al.*, 1969) or of antigen-sensitive cells (Moller & Zukoski, 1968).

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