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CONTACT SENSITIVITY IN ALLOXAN-DIABETIC MICE

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SUMMARY

Alloxan-diabetic mice of Swiss, CBA and DBA/2 strains show a significant depression of contact sensitivity to oxazolone, as compared with normoglycaemic control animals, which is accompanied by the involution of the thymus and spleen. Insulin treatment partially restores the contact sensitivity in diabetic animals and also increases the weight of lymphatic organs. In contrast, the non-specific inflammatory response to oxazolone is not impaired in insulin-deficient mice. Further experiments have shown that neither sensitized lymphocytes of control animals given to diabetic mice, nor sensitized lymphocytes of diabetic mice injected into normoglycaemic recipients, were able to transfer passively any significant contact sensitivity. It is suggested that in alloxan-diabetic mice the function of T lymphocytes is affected.

INTRODUCTION

When alloxan monohydrate is injected into various laboratory animals, destruction of insulin-secreting β cells in the islets of Langerhans occurs, while α cells are resistant to alloxan (Ruangsiri, 1947). Disappearance of β cells within a few days is accompanied by typical and permanent diabetes. Alloxan-treated animals may be considered as laboratory models of diabetes mellitus in man (Webb, 1966). Since in these animals the blood glucose level can be deliberately adjusted, they offer a useful experimental system for following the interrelationships between disturbances of carbohydrate metabolism and the ability to form an immune response. This paper presents evidence that in alloxan-diabetic mice there is a profound impairment of lymphocyte functions.

MATERIALS AND METHODS

Animals

Closed colony bred Swiss and inbred CBA and DBA/2 mice of both sexes, weighing 22–25 g were used, except in transfer experiments, when mice of only one sex were used.

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Induction of alloxan diabetes

A freshly prepared 0·75% solution of alloxan (Merck) was given intravenously (75 mg/kg). Mice were fasted for 24 hr prior to injection. Only mice with a blood glucose level exceeding 250 mg/100 ml were used in transfer experiments. Usually mice were used for experiment 4 days after alloxan administration. The mortality of diabetic animals during the experiment did not exceed 5%. Mice showing the signs of diabetic disease (diminished activity, dehydration) were excluded from the experiment.

Sensitization and challenge with oxazolone

Animals were sensitized by the application of 0·1 ml of 3% oxazolone in absolute ethanol to the skin of the clipped abdomen. Seven days later the mice were anaesthetized, the thickness of the ear was measured with an engineer's micrometer, and both sides of the ear were smeared with the 1% solution of oxazolone in olive oil, unless otherwise stated. The ear was measured after 24 hr and the increase of thickness expressed in units of 10^{-3} cm (Asherson & Ptak, 1968).

Passive transfer

For transfer experiments normal or alloxan-diabetic animals sensitized 8 days previously were used. Mice were injected intraperitoneally 4 days before transfer with 2 ml of heavy liquid paraffin. Peritoneal exudate cells were flushed out from the peritoneal cavity with 4 ml of phosphate-buffered saline containing 20 u of heparin. Inguinal, axillary and mesenteric lymph nodes were gently teased out into the cold 199 medium supplemented with 10% mouse serum. Cells were filtered through 110 N Nybolt gauze. A volume of 1 ml of mixed peritoneal exudate cells and lymphocytes suspended in 10% immune serum was injected intravenously into normal or alloxan-diabetic recipients. The mice were challenged within 1 hr. Cell viability was assessed by the Trypan Blue exclusion test. In control animals the lymph node cells were 75–85% viable, whereas the peritoneal cells were about 95% viable; in alloxan-diabetic mice the corresponding values were 60% and 90%. The number of transferred cells refers to viable cells.

Thymus and spleen weight assay

In groups of diabetic and control Swiss mice the spleens and thymuses were removed and weighed to an accuracy of 1 mg.

Insulin treatment

Alloxan-diabetic mice received 0·75 i.u. of insulin subcutaneously every 12 hr (Insulin Ultralente, Polfa), beginning from the day of sensitization up to the end of experiment. The mortality rate of insulin-injected animals was approximately 20%, presumably due to hypoglycaemia.

Blood glucose level

This was estimated by the *o*-toluidine method (Hyvarinen & Nikkilä, 1962) in the animals fasting for 12 hr. In insulin-treated animals the estimations were made 3 hr after drug administration.

RESULTS

Effects of alloxan administration before or after sensitization on contact sensitivity

Mice injected with alloxan, sensitized with oxazolone 4 days later, and tested after another 7 days, showed a diminished contact sensitivity as compared with normoglycaemic control animals (Table 1). It was also observed that the response to the challenging dose of oxazolone

TABLE 1. Contact sensitivity in mice made diabetic before or after sensitization

Mouse strain	Alloxan administered at day*	Ear swelling (mean ± s.d.)	Blood glucose level range (mg/100 ml)	Number of animals
Swiss	−4	3·9 ± 1·04	280–510	15
	+4	7·7 ± 2·10	310–420	12
	Positive controls	13·6 ± 3·21	60–120	6
	Negative controls	2·9 ± 0·32	60–100	6
DBA/2	−4	7·4 ± 1·86	250–380	15
	Positive controls	16·7 ± 2·08	40–100	8
	Negative controls	2·1 ± 1·00	50–110	6
CBA	−4	8·3 ± 1·11	260–400	8
	Positive controls	15·3 ± 1·98	40–120	5
	Negative controls	4·2 ± 0·75	60–100	6

* Mice were injected with alloxan (−4), sensitized with oxazolone 4 days afterwards, and tested 7 days later. Alternatively, animals were sensitized, injected with alloxan on day +4 and tested after another 4 days. Positive controls refers to ear swelling in normal sensitized mice, whereas negative controls is the non-specific ear swelling in non-sensitized normal mice.

roughly parallels the blood glucose level (Table 2). In other experiments mice were sensitized to oxazolone, injected with alloxan 4 days later and tested after a further 4 days. As shown in Table 1 this treatment also led to a significantly lower response to oxazolone. Since alloxan excretion and degradation proceeds very rapidly (Webb, 1966), it was concluded that the observed effects were not due to a direct effect of the drug itself. Table 2 demonstrates also that diabetic mice show a marked decrease in the weight of the lymphatic organs, which parallels the blood glucose level.

Non-specific ear swelling in alloxan-diabetic mice

When alloxan-diabetic mice and normoglycaemic control animals were painted with increasing concentrations of oxazolone in olive oil, the non-specific increment of ear thickness was identical in control and diabetic animals over the whole range of blood glucose levels (Table 3).

Passive transfer of contact sensitivity by cells of sensitized alloxan-diabetic mice

Table 4 demonstrates that peritoneal exudate and lymph node cells obtained from diabetic mice 8 days after sensitization with oxazolone and injected intravenously into normal recipients were entirely unable to transfer contact sensitivity.

TABLE 2. Response to oxazolone and weight of lymphatic organs in diabetic mice matched for blood glucose level

Blood glucose level range (mg/100 ml)	Number of animals	Ear swelling (mean ± s.d.)	Mean spleen weight (g)	Mean thymus weight (g)
150–250	12	14·1 ± 2·53	251	50
251–350	39	8·4 ± 1·13	220	41
351–500	36	6·7 ± 1·36	155	34
501 and more	34	4·1 ± 0·84	124	22
Positive controls	6	12·3 ± 3·14	270	66
Negative controls	6	2·8 ± 0·91	241	60

Swiss mice were injected with alloxan, 4 days later sensitized to oxazolone, and then tested 7 days later. Animals were matched in groups according to the blood glucose level. The weights of the spleens and thymuses were recorded to the accuracy of 1 mg.

TABLE 3. Non-specific ear swelling in alloxan-diabetic Swiss mice after painting with oxazolone

Concentration of oxazolone (%)	Ear swelling (mean ± s.d.)			
	Control mice Blood glucose level range (mg/100 ml)		Alloxan diabetic mice Blood glucose level range (mg/100 ml)	
	40–120	251–350	351–500	501 or more
1	2·9 ± 1·34	2·3 ± 0·88	2·5 ± 1·42	1·3 ± 0·70
2	3·3 ± 1·10	3·8 ± 1·21	3·8 ± 0·75	4·0 ± 0·92
5	8·3 ± 1·35	8·3 ± 1·24	9·3 ± 2·37	9·0 ± 3·02

Swiss mice were injected with alloxan. Groups of mice matched for blood glucose level were painted with different concentrations of oxazolone in olive oil. The non-specific ear swelling was recorded 24 hr afterwards. Each group consisted of five to twenty animals.

Passive transfer of contact sensitivity into alloxan-diabetic recipients

When peritoneal exudate and lymph node cells of normal, sensitized animals were injected either into normal or diabetic recipients, only normal recipients showed a significant ear swelling upon challenge with oxazolone, while diabetic animals failed to respond significantly. The differences between groups were more pronounced in DBA/2 mice (Table 4).

Influence of insulin administration on the development of contact sensitivity in diabetic mice

Diabetic sensitized mice treated with insulin showed in contrast to non-treated diabetic animals a significantly increased response to oxazolone. In these animals the weight of the lymphatic organs was also increased (mean values correspondingly: thymus, 33 mg and 46 mg; spleen, 220 mg and 270 mg). The schedule of drug administration used, however, does not completely restore the ability to respond to the challenging dose of contact sensi-

TABLE 4. Passive transfer of contact sensitivity

Type of transfer	Ear swelling (mean ± s.d.) Strain of mice: DBA/2	CBA experiment 1	CBA experiment 2
Normal donor→ Diabetic recipient	0·5±0·31 (6) [390]	4·2±1·27 (7) [335]	4·6±0·91 (6) [310]
Diabetic donor→ Normal recipient	1·7±0·46 (6)	3·5±1·29 (6)	3·8±1·02 (5)
Normal donor→ Normal recipient	9·1±2·44 (6)	7·6±1·16 (6)	8·0±2·21 (6)
Positive controls	17·0±3·07 (6)	15·4±2·00 (5)	13·7±2·14 (6)
Negative controls	1·1±0·87 (6)	2·7±0·81 (5)	2·3±0·13 (6)
Number of cells transferred			
Lymph node cells	$1·2\times10^8$	$7·0\times10^7$	$1·1\times10^7$
Peritoneal exudate cells	$1·5\times10^7$	$3·0\times10^6$	$1·0\times10^7$

Suspensions of lymphocytes and peritoneal exudate cells of control or diabetic mice sensitized to oxazolone 8 days previously were injected alternatively into normal or diabetic recipients. Mice were challenged with oxazolone within 1 hr and the ear swelling read 24 hr later. Animals sensitized 7 days previously, and non-sensitized mice were also included in the experiment as positive and negative controls respectively. The number of animals is given in parentheses and the mean glucose level in diabetic recipients is shown in brackets.

TABLE 5. Influence of insulin administration on the restoration of contact sensitivity in diabetic Swiss mice

Group	Ear swelling (mean ± s.d.) Experiment 1 (number of mice)	Experiment 2 (number of mice)
Diabetic mice	3·9±0·82 (9)	2·5±1·82 (8)
Diabetic mice+insulin	8·7±2·38 (9)	8·7±3·67 (6)
Positive controls	14·9±3·68 (7)	12·9±3·80 (6)
Positive controls+insulin	13·9±2·75 (5)	n.d.
Negative controls	2·70±0·92 (5)	2·1±1·04 (5)
Negative controls+insulin	2·8±0·50 (4)	n.d.

Swiss mice injected with alloxan 4 days previously and control mice were sensitized to oxazolone. Some diabetic and control mice, also non-sensitized, were injected every 12 hr with 0·75 u of insulin. The contact sensitivity was read 7 days after sensitization. The blood glucose levels (range in mg/100 ml) were as follows (experiment 1): diabetic mice 280–390; diabetic mice+insulin 80–190; control mice 40–110; control mice+insulin 10–40. The number of animals is shown in parentheses.

tizer. Insulin has no influence on the magnitude of response in normoglycaemic animals (Table 5).

DISCUSSION

The role of insulin in the metabolic activity of the lymphocyte is not entirely clear. Human lymphocytes have been shown to possess specific insulin binding sites on their surface (Gavin, Buell & Roth, 1972; Gavin *et al.*, 1973). This is presumably true also for lymphocytes of other mammals. Insulin stimulates plasma membrane ATPase activity (Hadden *et al.*, 1972), and glucose transport into lymphocytes (Boyett & Hofert, 1972), particularly of thymic origin. It also influences the uptake of aminobutyric acid by these cells (Goldfine, Gardner & Neville, 1972). While the significance of these metabolic processes for the immune response is not yet known, our data support the assumption that the saturation of receptor sites by insulin may be critical for the triggering of some biologically significant events in the lymphocyte.

Our experiments clearly demonstrate that in alloxan-diabetic mice there is a significant depression of contact sensitivity, which roughly parallels the blood glucose level and the involution of lymphatic organs. These interrelationships may reflect the different degrees of damage of insulin producing β cells, produced by alloxan. In contrast, the non-specific inflammatory response seems not to be impaired in diabetic animals. Insulin administration partially restores the response to oxazolone in diabetic animals, and also increases the weight of the thymus and spleen. A similar increase has also been reported in intact rats treated with insulin (Lundin & Angervall, 1970). Although the decreased ability of diabetic mice to mount the cell-mediated response may be due to regressive changes in the lymphoid organs, this cannot account solely for the effects observed since the transfer experiments have shown that: (i) sensitized lymphocytes of normoglycaemic animals passively transferred evoke a significantly lower contact reaction in diabetic mice than when given to normal recipients; (ii) lymphocytes of sensitized diabetic mice confer on normal recipients only a marginal ability to respond to the contact sensitizer. This implies that both afferent and efferent arcs of the cell-mediated response are affected, and the main cell suffering from insulin deficiency is presumably the T lymphocyte.

Our results are in line with other experiments in which it has been shown that delayed hypersensitivity to tuberculin is markedly depressed in alloxan-diabetic guinea-pigs (Thompson, 1961). Moreover, in rats sensitized to tuberculin the delayed reaction is only poorly expressed unless the animals are treated with insulin (Thompson, 1967). In contrast, the humoral response seems not to be influenced in alloxan-diabetic animals (Adamkiewicz, 1963; Dolkart, Halpern & Perlman, 1971).

The increased incidence of infections in human diabetic patients has as yet no satisfactory explanation. Contradictory results have been obtained when normal versus diabetic subjects were compared for their ability to produce antibodies (compare with Dolkart *et al.*, 1971). No depression of cellular immunity in diabetics has been so far reported. Since, however, any insulin deficiency is adjusted by appropriate administration of drugs, such high glucose levels as occur in animals are scarcely ever noted in human subjects. The possibility cannot, however, be entirely excluded that even in compensated cases of diabetes mellitus there is a decreased ability to form cell-mediated responses. Impaired lymphocyte function might be an adjunct to the well-known depression of phagocytosis (Bagdade, Root & Bulger, 1974) in causing the increased susceptibility of diabetic patients to many infections.

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