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Prevention of Acute Immunological Lung Lesion in Rats by Decomplementing Treatment

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Summary. The intravenous administration of nephrotoxic antibody serum to rats produced a rapid and pronounced reduction in the serum complement level; this was observed before lung lesions became apparent.

A total suppression of the acute immune lung change was observed in animals depleted of complement by treatment with heat-aggregated human γ-globulin or zymosan.

Albeit the experimental evidence presented is of indirect nature, it suggests that the complement system is involved in the mediation of the acute pulmonary injury following injection of nephrotoxic antibody serum.

INTRODUCTION

It is well known that serum complement (C′) can play a fundamental role in different types of lesions induced by immunological reactions (evidence reviewed by Osler, 1961; Nelson, 1965; Lepow, 1965).

Previous reports from this laboratory have shown that the administration to the rat of nephrotoxic antibody serum (NTS) in appropriate amounts is rapidly fatal; one of the most striking features of this '*in vivo*' antigen–antibody interaction is an acute haemorrhagic and oedematous lung change (Laus-Filho and Hadler, 1960; Oliveira and Laus-Filho, 1962).

In determining the specific mediators which operate in this peculiar class of immunopathological lesion the participation of the C′ system deserves attention, since it has been frequently verified that depression of serum C′ occurs in the course of immune reactions *in vivo* (Seltzer, Baron and Fusco, 1952), particularly after the administration of NTS (Stavitsky, Hackel and Heymann, 1954; Pfeiffer, Schöffling, Bruch and Spielmann, 1954; Lange, 1954; Earle, 1959; Unanue and Dixon, 1964).

The data reported here show that a resistant state against the acutely lethal effect of NTS is developed in rats rendered C′ deficient by treatment with heat-aggregated human γ-globulin (HAHGG) or zymosan.

MATERIAL AND METHODS

Animals

Female rats of Wistar strain weighing 80–90 g were used throughout the study.

Nephrotoxic serum (NTS)

This was prepared from rabbits hyperimmunized with rat lung or rat kidney homogenates as previously described (Oliveira, Laus-Filho and Sarti, 1966). The anti-lung and anti-kidney sera were pooled and the pool was assayed to determine the dose required to cause severe lung change and death in most normal rats within 10 minutes of injection. With the animals used in the experiments reported here the effective dose was 0·35 ml/rat. Except where otherwise stated, rats surviving for 30 minutes after NTS injection were then killed and examined.

Lung changes

The lungs of all the experimental animals were examined after death, and the lung lesions scored as *severe*, *moderate* and *absent*, as described elsewhere (Oliveira, Laus-Filho, Sarti and Carvalho, 1967).

Decomplementation procedures

(a) *Heat-aggregated human γ-globulin (HAHGG)*. Human γ-globulin (Cohn's Fraction II) in solution of 25 mg/ml was heat-aggregated at 62° for 20 minutes. In most experiments the decomplementing injection of 1·2 mg of HAHGG was administered intravenously 15 minutes before the challenge with NTS.

(b) *Zymosan (Fleischmann Laboratories)*. Boiled zymosan in 0·15 M NaCl (15 mg/ml) was given as follows: 15 mg (or 5 mg) intraperitoneally + 10 mg (or 3 mg) intravenously 30 and 15 minutes, respectively, before the injection of NTS.

Analysis of complement levels ($C'H_{50}$)

It was not found possible to aspirate blood after the pulmonary lesion had been induced. Therefore the animals challenged with NTS intravenously were bled 3 minutes after the injection, before there was any evidence of macroscopic lung change. Bleeding was done by cardiac puncture with the thorax open. The collected blood was allowed to clot at room temperature and then spun in a refrigerated centrifuge. The sera were transferred to test tubes and maintained on crushed ice until titrated for C′: this was performed as described by Almeida (1950). $C'H_{50}$ values for normal female rats weighing 80–90 g ranged from 140 to 300 U/ml.

Platelet depletion

This was carried out using lauric acid treatment as described by Zbinden (1967). Lauric acid solution (5·0 mg/ml) was adjusted to pH 8·5 with N NaOH and 2 mg were injected intravenously 15 minutes before the administration of NTS.

Haematological methods

The white blood cells were counted with the Neubauer chamber and the platelets by the method of Brecher and Cronkite (1950).

RESULTS

PREVENTION OF LUNG LESIONS BY C′ DEPLETING PROCEDURES

The data presented in Table 1 show that the pretreatment of rats by HAHGG or zymosan results in a marked protection of the animals against the pathogenetic action of NTS. Death following the intravenous injection of NTS occurred in nine of ten control

(untreated) animals and severe lung changes were present in all rats of this group. By contrast, the animals pretreated with HAHGG (20 mg down to 1·2 mg/rat) or zymosan (25 mg/rat) were totally insensitive to the acute effects of NTS and no deaths or lung lesions were observed. The degree of protection was decreased as the amount of HAHGG or zymosan was reduced. The animals of the group pretreated with 0·5 mg HAHGG or 8 mg of zymosan showed lung changes and in the rats pretreated with 0·3 mg of HAHGG one death occurred. It is clear from Table 1 that the minimum amount of HAHGG injected which afforded complete protection in the stated experimental conditions was 1·2 mg/animal.

TABLE 1

INCIDENCE OF DEATHS AND LUNG CHANGES IN DECOMPLEMENTED RATS CHALLENGED WITH 0·35 ml OF NTS ADMINISTERED INTRAVENOUSLY

Groups of rats		'Decomplementing' treatment		No. of deaths*	Macroscopic lung changes		
					Absent	Moderate	Severe
Controls							
Injected with NTS only	(10)	None		9	0	0	10
Pretreated							
Injected with HAHGG 15 minutes before NTS	(3)	20 mg	HAHGG i.v.	0	3	0	0
	(3)	10 mg		0	3	0	0
	(3)	5 mg		0	3	0	0
	(3)	2·5 mg		0	3	0	0
	(3)	1·2 mg		0	3	0	0
	(3)	0·6 mg		0	1	1	1
	(3)	0·3 mg		1	0	2	1
Injected with zymosan before challenge by NTS†	(3)	15 mg i.p. + 10 mg i.v.		0	3	0	0
	(3)	5 mg i.p. + 3 mg i.v.		0	1	2	0

Figures in parentheses indicate the number of animals.

* Deaths due to acute pulmonary oedema within 30 minutes from the intravenous injection of 0·35 ml of NTS. The surviving animals were killed at the end of 30 minutes.

† Intraperitoneal and intravenous injections 30 and 15 minutes, respectively, before the injection of NTS.

EFFECT OF ADMINISTRATION OF NTS AND TREATMENT WITH HAHGG ON SERUM C′ ACTIVITY

Rats were injected intravenously with 0·35 ml of NTS, an amount sufficient to cause death with acute pulmonary oedema within 10 minutes in most animals. The serum C′ level in the blood collected 3 minutes after the challenging injection of NTS was markedly reduced and only three of nine animals had some detectable activity (Fig. 1). In an attempt to determine the intensity of the decomplementing procedure, three experimental groups of rats were used for C′ depleting studies. The selected doses of HAHGG were 5, 1·2 and 0·3 mg/rat for each group, respectively. It can be seen from Fig. 1 that the group injected with 0·3 mg of HAHGG did not show any significant alteration of serum C′ activity when compared with the untreated animals. The same was not true for the other groups. It is worth while to note that the injection of 1·2 mg of HAHGG, which provided complete protection against the acute effects of NTS, was also sufficient to induce a small but definite depression on the serum C′ activity level.

DEVELOPMENT OF LUNG CHANGES PROVOKED BY NTS ADMINISTRATION AT DIFFERENT TIME INTERVALS AFTER THE C′ DEPLETING INJECTION

Experiments were performed to determine the duration of the state of refractoriness to the action of NTS in decomplemented rats. The data presented in Table 2 reveal that the

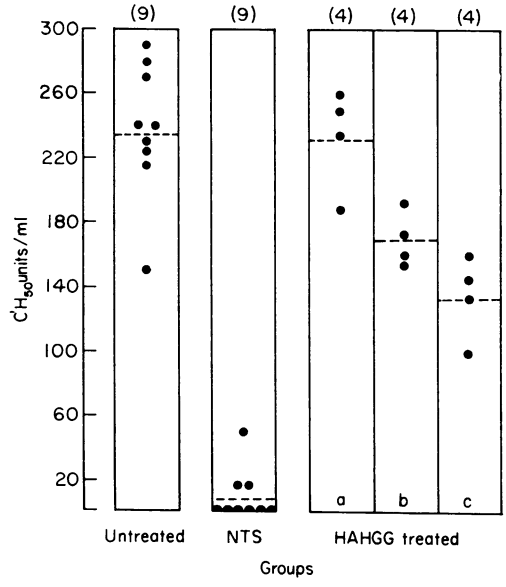

FIG. 1. Reduction of the serum C′ titre in rats challenged with NTS or treated with HAHGG. The NTS group received 0·35 ml of NTS intravenously 3 minutes before blood collection. The HAHGG group was treated with 0·3 mg(a), 1·2 mg(b) or 5·0 mg(c) of HAHGG intravenously 15 minutes before bleeding. Figures in parentheses indicate the number of animals in each group.

C′ depletion produced by injection of 1·2 mg of HAHGG afforded complete protection against the action of NTS within the first 30 minutes after the decomplementing injection. In the group depleted of C′ 1 hour before the injection of NTS moderate lung changes in two out of three animals were observed. When the challenging NTS was administered 2 hours after the decomplementing injection severe lung lesions and deaths occurred.

Further data in Table 2 show that the injection of 1·2 mg of HAHGG simultaneously with NTS totally failed to prevent the development of pulmonary changes.

TABLE 2

OCCURRENCE OF DEATHS AND LUNG CHANGES IN RATS DECOMPLEMENTED AT DIFFERENT TIME INTERVALS BEFORE THE ADMINISTRATION OF NTS

Groups of rats		Time elapsed after 'decomplementing' injection†	No. of deaths*	Macroscopic lung changes		
				Absent	Moderate	Severe
Treated						
Injected with 1·2 mg HAHGG	(3)	5 minutes	0	3	0	0
before NTS	(3)	15 minutes	0	3	0	0
	(3)	30 minutes	0	3	0	0
	(3)	1 hour	0	1	2	0
	(3)	2 hours	2	1	0	2
	(6)	Simultaneous	5	1	0	5
Controls						
Only NTS injected	(3)	–	3	0	0	3

The figures in parentheses indicate number of animals.

* Correspond to the time interval between the administration of HAHGG and challenge by 0·35 ml of NTS.

† Deaths due to acute pulmonary oedema within 30 minutes from the i.v. injection of 0·35 ml of NTS. The surviving animals were killed at the end of 30 minutes.

EFFECT OF DECOMPLEMENTING TREATMENT WITH HAHGG ON LEUCOCYTES AND PLATELETS

Since the C′ depleting procedure could interfere with leucocyte and platelet numbers, experiments were carried out to determine whether this was the case in our studies. In order to allow for the variability of white cell and platelet counts, the rats were bled and counts performed 24 hours, 18 hours and 15 minutes before the C′ depleting injection. No consistent changes were observed in these controls. Leucocyte counts did not vary significantly after the injection of 1·2 mg of HAHGG, while platelet counts showed a slight tendency to decrease after the injection. Fig. 2 depicts a representative experiment in which only counts performed 15 minutes before and 15 minutes after the challenging injection were considered.

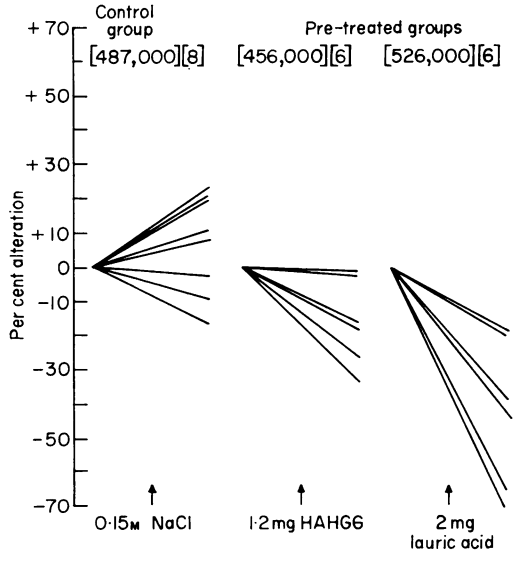

FIG. 2. Platelet counts in controls and rats treated with HAHGG or lauric acid. In all animals the blood was collected 15 minutes before and 15 minutes after the intravenous injection of saline, HAHGG or lauric acid. Figures in parentheses indicate the initial mean value for platelets per mm^3 of blood, followed by the number of rats in each group. The arrows indicate the i.v. injection.

EFFECT OF PLATELET DEPLETION ON THE DEVELOPMENT OF LUNG CHANGES

To find out whether or not platelet depletion was essential for the establishment of refractoriness to the action of NTS, rats rendered thrombocytopenic with lauric acid were subsequently challenged with NTS. The intravenous injection of 2 mg lauric acid caused some prostration of the rats, usually followed by prompt recuperation. The platelet depletion resulting from this treatment was compared to that observed in the animals treated with HAHGG, as shown in Fig. 2. Although lauric acid *per se* can produce pulmonary thrombosis, no detectable macroscopic lesions were noticed in control rats treated with 2 mg of this drug. The platelet depleted rats were as sensitive to NTS challenge as untreated animals. Thus, all thombocytopenic animals developed severe lung change when injected with NTS (Table 3).

TABLE 3

DEVELOPMENT OF LUNG LESIONS IN PLATELET-DEPLETED AND CONTROL RATS AFTER CHALLENGE BY NTS

Groups of rats		No. of deaths*	Macroscopic lung changes		
			Absent	Moderate	Severe
Controls Injected with NTS only	(3)	3	0	0	3
Controls Injected with 2 mg of lauric acid only	(6)	0	6	0	0
Treated Injected with 2 mg of lauric acid 15 minutes before NTS	(9)	8	0	1	8

Figures in parentheses indicate number of animals.

* Deaths due to acute pulmonary oedema within 30 minutes from the i.v. injection of 0·35 ml of NTS. The surviving animals were killed at the end of 30 minutes.

DISCUSSION

The acute lung lesion under investigation results from the reaction of passively-administered heterologous antibody against antigenic constituents of the host lung tissue, and can thus be classed, in the scheme of Coombs and Gell (1963) as a Type II reaction. Complement is known to be involved commonly in the cytolytic or cytotoxic effects of this type of reaction. It is clear from our experiments that injection of NTS into normal rats resulted in severe and rapid fall in serum complement activity. This observation agrees with the early report of Stavitsky *et al.* (1954), who also demonstrated that the drop in C′ could not be attributed exclusively to the reaction between NTS and the kidney, since it was shown that even in bilaterally nephrectomized rats the injection of NTS still led to a decrease of serum C′. This finding could be explained as resulting from extra-renal tissue antigen–antibody interactions caused by the known cross-reactivity of the NTS with a variety of tissues, including the lung (Pressman and Sherman, 1951). Furthermore, the pulmonary-injuring property of NTS is completely lost from sera previously absorbed with rat lung tissue (Laus-Filho and Hadler, 1960).

The rapidity of the fall in C′ level was impressive, no detectable C′ activity being observed in most animals within 3 minutes after the injection of NTS. If fixation of C′ is a prerequisite for the production of the lesion, inhibition of the development of the lung changes should result from an unrelated decomplementing procedure. This was observed in rats decomplemented by treatment with HAHGG or zymosan, in which the injection of NTS was completely devoid of lung damaging activity. Moreover this immunological insult appeared highly sensitive to the availability of C′. A reduction of 25 per cent in the serum C′ level was accompanied by a state of total refractoriness to the acute effects of NTS. The relative smallness of the decrease of serum C′ level that in some animals afforded complete protection should not be underevaluated. It is known that the overall C′ level does not necessarily indicate the actual concentration of individual C′ components. Therefore, a slight reduction of the haemolytic C′ titre might be associated with a severe depression of a C′ component essential for its tissue injuring activity.

The amount of HAHGG necessary to establish insensitivity to the action of NTS is strikingly smaller than that reported to decrease delayed hypersensitivity (Neveu and Biozzi, 1965) and to inhibit the Arthus reaction (Ward and Cochrane, 1965), even when taking into account the differences in the weight of the rats used in our and their experiments. However, it must be taken into consideration that the immune lung change

provoked by NTS is a very acute one, developing within a few minutes, in contrast to the above cited phenomena. Furthermore, the protective effect of treatment with 1·2 mg of HAHGG is promptly manifested, being evident in five minutes and lasting for only 30 minutes, after which the animals regained sensitivity to NTS. It seems reasonable to suppose that the short duration of the resistant state promoted by the above treatment is linked at least in some degree to the partial depletion of serum C′ activity. No attempts were made to explore the effectiveness of larger doses of HAHGG in prolonging the resistant condition.

We have shown previously that this type of immunological lesion is completely inhibited in rats rendered uraemic (Oliveira *et al.*, 1966) or treated with a substance extracted from the urine of normal animals (Oliveira *et al.*, 1967). The earlier demonstration that uraemic rats insensitive to the action of NTS had normal serum C′ levels, poses an intriguing question. At this point it is very difficult to provide a single explanation of the refractory states to NTS in animals rendered either C′ deficient or uraemic (but with C′ unchanged). The possibility that in uraemic rats the utilization of C′ is impaired cannot be ruled out. It is hoped that further studies dealing with the rate of depletion of C′ in uraemic animals challenged by NTS may settle this problem. Mention should be made of the observed failure to prevent lung changes by the injection of HAHGG simultaneously with NTS. This differs from the observed properties of the urinary fraction which has the ability to block the action of NTS when administered simultaneously (Oliveira *et al.*, 1967). It should be emphasized, however, that this effect was studied with a partially purified urinary substance and no particular effort was made to determine whether the dose employed was larger than the minimum effective one. Recent work from this laboratory on the resistant state induced by the urinary fraction has shown a decomplementing effect of this material. Furthermore, when compared to HAHGG on a basis of minimum protective dose, the degree of C′ depletion provoked by both substances is quite similar (Carvalho, Oliveira, Laus-Filho and Sarti, unpublished).

Considering the above findings, i.e. the high degree of protection given by two procedures known to induce decomplementation, and the protective action exhibited by the urinary fraction with concomitant C′ depletion, it is reasonable to suspect that complement plays a causal role in the lung insult provoked by NTS. Unfortunately, the modifications induced by C′ depleting methods *in vivo* are not yet fully understood, thus making it impossible at present to exclude other interpretations. The decomplementing treatment might in some way adversely affect the activity of natural mediators mobilized in the course of the immune reaction; if so, the data herein reported could represent an event incidental and irrelevant to the actual mechanism of the lesion. Bearing on this question is the demonstration by Osler, Haurisiak, Ovary, Siqueira and Bier (1957) that decomplementation of albino rats did not modify the responses of histamine liberators. Studies on other pharmacological mediators are still needed, but at least two of the known associated effects—leucopenia and thrombocytopenia—of C′ depleting treatment are not operating in our experimental conditions. With the dosage of HAHGG used, only a slight fall in the platelet count was noted and was this not significant to the mechanism of resistance developed in C′ depleted animals, for the more severe thrombocytopenia induced by lauric acid failed to prevent the immune lung change.

It is pertinent to recall here the results of the elegant experiments of Hammer and Dixon (1963) on the participation of C′ in nephrotoxic serum nephritis, an immunologically mediated lesion very similar to ours. As they have shown, decomplementation

induced before the administration of rabbit NTS prevents the development of the immediate glomerular injury.

On balance, the results of our experiments may be regarded as evidence that the C′ system is involved in the mediation of the lung-injuring effect of NTS. Moreover, the efficiency of zymosan as a protective agent suggests that participation of C′3 is essential to the development of the lung lesion. Germane to this are the studies of Osler *et al.* (1957), implicating the participation of complement in passive cutaneous anaphylaxis (PCA) in the rat, with possible formation of anaphylatoxin (Osler, Randall, Hill and Ovary, 1959). Thus, as is supposed in PCA, the acute lung change in rats appears to result, at least in part, from a damaging mechanism involving the participation of the C′ system. If so, this kind of immune-dependent lesion will be a sensitive and an appropriate model for further investigations on the questions of the direct tissue injury mediation by complement *in vivo*.

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