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Protection of Mice Against Group B *Streptococcus* Type Ia by IgG Components of a Rabbit Antiserum

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Abstract. IgG fractions were separated by ion exchange chromatography on DEAE-cellulose from hyperimmune rabbit sera prepared against a group B *streptococcus* type Ia mouse-virulent strain. 50 μg IgG in conjunction with ampicillin (200 mg/kg) protected mice more effectively against a lethal challenge than ampicillin (400 mg/kg) alone or ampicillin (200 mg/kg) combined with gentamicin (10 mg/kg), when administered up to 12 h after infection.

Introduction

Bacterial infection in neonates frequently responds to antibiotic treatment, but in some cases the administration of even the most potent antibiotics is not successful [12].

Shigeoka [13] and Gamsu [5] have pointed out the desirability of augmenting the demonstrably poor defences of the infant by the administration of, for example, specific immunoglobulins. Previously it has been shown that immune whole serum will protect experimental animals against a group B streptococcal infection [1, 6, 14], but so far there are no reports of more refined gammaglobulins, which woud be clinically more acceptable, having been used. The experiments in this presentation were carried out, therefore, to investigate the possibility that IgG combined with an antibiotic might be a more suitable method of treating group B streptococcal disease at various times after the infection had become established.

Materials and Methods

(a) Mouse-virulent Group B Streptococcus

A type Ia strain, isolated from a pregnant women, was passaged through adult female white mice (CRC/TO), weighing 26–28 g [6]. After 25 passages it was found that intraperitoneal injection of approximately 4.0×10^5 colony forming units (cfu) of the streptococci, grown for 18 h in Todd-Hewitt broth at 37°C, resulted in the death of ten out of ten 21-day-old mice (LD100) within 5 days.

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(b) Preparation of IgG Fractions

Hyperimmune rabbit antiserum against the mouse-virulent group B streptococcal strain was raised in adult New Zealand white rabbits by the method of Lancefield et al. [6], heat inactivated at 56°C for 30 min and absorbed with Ib cells to produce type-specific Ia sera. Ouchterlony analysis, slide agglutination, and an enzyme-linked immunosorbent assay [3] were carried out on the serum to assess antibody titre.

Ion exchange chromatography on DEAE-cellulose was carried out on the hyper-immune serum and on normal rabbit serum to be used as control, by the method of Williams and Chase [15] with modifications from that of Levy and Sober [8]. Sodium or ammonium sulphate precipitation was not carried out on the serum before addition to the column, since these salts tend to produce aggregated IgG, which adheres to the column. Fractions from the column were assayed for total protein by the method of Bradford [2] and concentrated using Sartorius ultrafiltration equipment.

IgG in the fractions was detected and assayed by Laurell two-dimensional immunoelectrophoresis [7] and Mancini single radial immunodiffusion [9]. The first fraction contained IgG in a relatively pure form, but more anodal IgG was also detected in fractions 2 and 3. Extractions of IgM and IgA were not attempted due to their very low concentrations in rabbit serum and difficulty in isolation [11].

(c) Mouse Protection Tests

Groups of 21-day-old female mice (CRC/TO) were injected intraperitoneally with approximately 4.0×10^5 cfu streptococci in 0.1 ml phosphate-buffered saline (PBSA), and intramuscularly with 0.1 ml antibiotics or a combination of antibiotics and IgG. Controls consisted of groups of mice injected with normal whole serum, normal IgG fractions, bacteria alone, and PBSA alone. Animals were examined at least once daily, and moribund mice were immediately killed. No animals died after the 5th day following injection of the streptococci.

Results

The first series of experiments consisted of injecting groups of mice I.P. with a lethal dose (4.0×10^5 cfu in 0.1 ml PBSA) of the mouse-virulent group B streptococci type Ia, to which had been added 0.1 ml hyperimmune serum immediately before injection of the mixture. Control groups of mice were injected with the same dose of streptococci mixed with 0.1 ml normal serum.

The mice injected with the bacteria in hyperimmune serum survived for the 5-day observation period, even at a dilution of 1/100, whereas the mice injected with bacteria in undiluted normal serum did not survive beyond the third day after injection.

In subsequent experiments groups of mice were injected intramuscularly with various concentrations of IgG and intraperitoneally with a lethal challenge of group B streptococci. It can be seen from Table 1 that each of the three IgG fractions protected all the mice when administered in 100 μg doses, and fraction F2 was less protective than fractions F1 and F3. The majority of mice given 100 μg of non-immune IgG fractions died from the infection.

Table 1. Protection of mice with hyperimmune IgG fractions (F) following intraperitoneal injection with group B streptococci type Ia (4.0×10^5 cfu)

IgG Fraction	IgG Content (μ g.)	No. mice injected	No. animals surviving
Hyperimmune:			
F1	100	4	4
	10	4	4
	1	4	0
F2	100	4	4
	10	4	0
	1	4	0
F3	100	4	4
	10	4	2
	1	4	2
Non-immune:			
F1	100	4	0
F2	100	4	0
F3	100	4	1

Penicillin or ampicillin, combined with gentamicin, are frequently used to treat group B streptococcal infection in the newborn, though this is not always successful [12]. In view of the protective effect of the IgG previously demonstrated, experiments were then designed to study the effect of administering IgG in conjunction with ampicillin at various times after infection, and to compare the efficacy of IgG treatment with that obtained by administration of antibiotics alone (see Table 2). It is apparent that the IgG fractions and ampicillin (200 mg/kg) conferred better protection on the mice than antibiotics alone, even though administered as long as 12 h after infection. However, none of the treatments are effective when there is a delay of 16 h between infection and the administration of the various substances.

Table 2. Protection of mice with antibiotics, and antibiotics + IgG following intraperitoneal injection of group B *Streptococci* type Ia (4.0×10^5 cfu)

Time of treatment post-infection (h)	No. mice injected	Number of animals surviving					Untreated controls
		Ampicillin (200 mg/kg) + Gentamicin (10 mg/kg)	Ampicillin (400 mg/kg)	Ampicillin (200 mg/kg) + IgG (50 μg)			
				F1	F2	F3	
1	4	4	4	4	4	4	0
2	4	4	4	4	4	4	0
4	10	10	10	10	10	10	0
6	10	6	6	10	10	9	0
8	10	2	4	9	9	8	0
10	10	1	4	8	8	4	0
	4	0	0	1	0	0	0
12	10	1	1	8	6	3	0
	10						
		1	1	9	9	5	0
Total	24	2	2	18	15	8	0
16	10	0	0	2	0	0	0

Discussion

The experiments in this presentation demonstrate that type-specific IgG, prepared from group B streptococcal antisera, protects against the lethal effect of an infection previously established in mice. Serum protection in mice has been well correlated with immunity to human infection with streptococci in the past. However, in clinical prac-

tice, both in human and veterinary medicine, it is well established that the administration of antibiotics, particularly the penicillins, allows highly efficacious forms of treatment against group B streptococci. Nevertheless, there are occasions when patients do not respond to antibiotic therapy [12]. The reasons for the failure to respond are not always clear, but it is recognised both clinically and experimentally that a delay of a few hours from the time of infection before administering antibiotics may render the treatment ineffective, particularly if the infecting organism is highly pathogenic [4, 10].

In the present series of experiments successful treatment of the mice with ampicillin or ampicillin and gentamicin was obtained if administered not later than 4 hours after infection. However, by giving immune gammaglobulin simultaneously with the antibiotic, survival was significantly increased up to 12 h after infection.

It is not possible from these experiments to draw any firm conclusions about the mode of action of the immunoglobulins. However, it seems probable that they have a significant and immediate beneficial effect on the host's defence mechanisms, possibly by opsonisation of the organisms with subsequent enhancement of phagocytosis. No direct killing of the streptococci by IgG was observed in vitro.

These findings relate to the efficacy of administration of IgG against a single serotype of group B streptococci adapted for mice. If the administration of immune gammaglobulins was to be used as an adjunct to current therapy, it would probably be necessary to have available immune gammaglobulins prepared against other serotypes of group B streptococci; the ideal preparation being the production of monoclonal antibodies.

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