Age-dependent presence of antibodies in rat dams, capable of conferring protection against group B Streptococcus infection in neonates

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Abstract

A rat model was used to investigate maternal age-dependent resistance on group B Streptococcus (GBS)-induced mortality of the offspring. Offspring from young (first time) or older (repeat litters) dams were challenged with GBS. There was an approximate log difference in the dose of GBS required to cause identical levels of mortality in the two groups. The sera of the dams from both groups were analysed by whole-cell ELISA, and it was demonstrated that sera from the older dams possessed circulating IgG cross-reactive to GBS. Since IgG is transplacentally transferred, we conclude that this is the method of observed protection. © 2001 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

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1. Introduction

Group B Streptococcus (GBS) infections are the most common cause of bacterial sepsis in human infants during the first week of life. Deficiency of circulating maternal anti-GBS antibody correlates with susceptibility to the disease in the newborn offspring [1]. Studies designed to develop vaccines against this disease require an efficient model system. Models which have been developed include mouse [2], primate [3] and rat [4].

While the mouse model has been used for active vaccination studies, the neonatal rat model lends itself to passive vaccination studies. Since protection in the human system is entirely due to transplacentally transferred IgG, a passive vaccination model is as effective as an active model. The neonatal rats can easily be fostered to different dams, enabling a greater degree of randomisation in studies than is possible with mice. Further, the size of the neonatal rats allows for immunisation and subsequent infection to be carried out at two different sites, thus ameliorating possible false-positive results due to opsonisation of the bacteria when injected into the same site as the immunisation.

As with the mouse model, the neonatal rats show an age-dependent susceptibility to the disease which is similar to the human condition [5]. Here we report that in addition to this neonatal effect, there is a maternal age-dependent susceptibility, correlating with a recent report on the increase in anti-GBS IgG in human mothers with increasing age [6].

2. Materials and methods

2.1. Bacteria

GBS of the type Ia/c serotype, strain A909, was obtained from the ATCC. Following streaking and selection of blood agar plates (Columbia agar with horse blood, Oxoid, Basingstoke, UK), a colony was confirmed as being GBS using an agglutination test (Oxoid). Bacteria were grown in Todd–Hewitt broth (THB) (Oxoid) at 37°C and used to create master and working cell banks. Samples from the working cell banks were plated onto blood agar plates and haemolysis observed prior to use in these ex-
periments. A colony from the blood agar plate was taken and used to inoculate a 10-ml culture in THB overnight. From this culture, 100 µl was used to inoculate a fresh 20-ml culture. After approximately 3 h, the OD$_{600}$ was read and the cells collected by centrifugation. Using an experimentally determined conversion factor, the cells were resuspended in phosphate-buffered saline (PBS) to a final concentration of between $1\times10^5$ and $2\times10^7$ cfu ml$^{-1}$. After inoculation into the animals, dilutions of the bacterial suspensions were made and plated out on blood agar plates and incubated overnight at 37°C. Colonies were counted to ensure that the dose given was as expected and that the culture used was pure.

2.2. Animals

Pregnant time-mated Crl:CD (SD) IGS BR rats were obtained from Charles River (UK) Laboratories. Animals from Charles River are supplied as VAF/PLUS status, and are routinely monitored for the presence of GBS. During the period of these studies, the presence of GBS was not noted. Two different groups of animals were obtained: young dams (70 days, first mating) and older dams (120–180 days, proven females), all of which originated from the same production unit. The animals were separately housed in individually ventilated polysulfone cages (Tecniplast, UK). Free access to food (RM3P from Special Diet Services, UK) and water was allowed. The animals were housed and maintained in accordance with the Code of Practice for the Housing and Care of Animals used in Scientific Procedures issued by the UK Home Office. The experiments were carried out under the authority of a Project Licence granted under the Animals (Scientific Procedures) Act 1986.

2.3. Procedure

Experiments with young and old dams were carried out at different times. Neonates (1–36 h old) were removed from the dams, randomised and marked. Groups of between 12 and 16 neonates were inoculated subcutaneously with 50 µl containing different doses of GBS. They were returned to the dams and observed at least four times daily for signs of terminal infection, in which case the animals were killed by a schedule I method. Blood was sampled and sera prepared from four young and four old dams whose offspring were not inoculated with GBS.

2.4. Whole-cell enzyme-linked immunosorbent assay (ELISA)

A 20-ml late-exponential-phase culture of GBS in THB was prepared by inoculating media from the working cell banks. The bacteria were collected by centrifugation and resuspended in 5 ml PBS. ELISA plates (Nunc Immuno-Sorb, Life Technologies Ltd., Paisley, UK) were coated with 50 µl of the bacterial suspension and allowed to coat overnight at 4°C. All subsequent incubations were for 1 h at 37°C. Plates were blocked with 3% bovine serum albumin in PBS. Rat sera were diluted 1:100 with PBS and used as a primary antibody. The secondary antibody was a 1:3000 dilution of alkaline phosphatase-conjugated goat anti-rat antibody (Calbiochem, CN Biosciences, Nottingham, UK). Plates were developed with p-nitrophenyl phosphate (Sigma, Dorset, UK) for 30 min at 37°C and immediately read at OD$_{405}$.

3. Results

3.1. Effect of maternal age on GBS-induced mortality

The difference in the number of GBS-induced fatal infections in neonatal rats from young and old dams was examined. Doses of between $5\times10^3$ and $1\times10^6$ cfu of serotype Ia/c GBS were administered to each pup subcutaneously and survival monitored over a 72-h period. Plating demonstrated that the cultures were pure and that the dose was within 0.05 log of that intended. Fig. 1 shows the effect of the maternal age on the percentage mortality for groups which were administered different doses of GBS. The younger the age of the dams, the greater the susceptibility of the pups to succumb to the GBS challenge.

3.2. Effect of maternal age on circulating cross-reactive GBS antibody

Blood samples were taken from four dams whose pups were not infected with GBS and serum was prepared. This

![Graph showing the effect of maternal age on GBS-induced mortality](FEMSLE 10057 1-8-01)
was used as a primary antibody in an ELISA assay against whole type Ia/c GBS. Initial experiments (data not shown) indicated that the most accurate results were obtained when the serum was used at a dilution of 1:100. Fig. 2 shows the results of the ELISA assay with the calculated mean and standard deviation for the four serum samples being shown. The response of the sera to GBS from the older dams is significantly higher than that from the younger dams (double $t$-test, $P<0.01$). There is no difference in response to bovine serine albumin-blocked plates, showing that the response is not due merely to an increased non-specific ‘stickyness’ of the sera from the older dams. Western blot analysis of outer surface preparations of type Ia/c GBS indicates that the activity in the sera of the old dams is chiefly directed against carbohydrate rather than a protein component such as the c-protein (data not shown).

4. Discussion

In order to set up meaningful animal models for vaccine development against GBS infection, it is essential that clinically relevant and robust models be used. This report demonstrates additional correlations between the neonatal rat model and the human condition above what has already been reported, and additionally points towards information which must be taken into account when using these (and possibly other murine) models in vaccine development.

It has been recently reported that there is an increase in the prevalence of anti-GBS IgG with increasing age in humans [7]. Interestingly, we have observed such an increase in female rats. Further, the offspring of the older female rats are more resistant to the effects of GBS than the offspring of younger females. These results demonstrate the value of the neonatal rat model for studies on GBS infection as a mimic of the human condition.

However, the presence of immunodominant and protective anti-capsular antibodies [8] may mask the potential protective effect of any applied vaccine treatment. As demonstrated in this research, despite the absence of GBS in a breeding colony, circulating antibodies cross-reactive to GBS can be present in the mother and these can offer protection to the neonate.

References