

ORIGINAL ARTICLE

A hypothesis: antenatal sensitisation to respiratory syncytial virus in viral bronchiolitis

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Aim: To investigate the possibility of antenatal sensitisation to respiratory syncytial virus (RSV).

Methods: A total of 36 cord blood specimens were obtained from newborn infants; serum IgA was measured to exclude maternal blood contamination. Cord peripheral blood mononuclear cells were separated and cultured in the presence of either uninfected negative control cells or cells containing ultraviolet (UV) inactivated RSV. Proliferation was assessed by tritiated thymidine incorporation. Supernatant cytokine concentrations were measured using ELISA.

Results: Significantly higher proliferative response rates to UV inactivated RSV were shown in those infants exposed in utero to the RSV epidemic after 22 weeks gestation. UV inactivated RSV stimulation induced significantly higher interferon γ production from specimens with a positive proliferative response (sensitised) than from those with a negative response (not sensitised).

Conclusions: Antenatal sensitisation to RSV occurs in one third of infants exposed to an RSV epidemic at the appropriate time of gestation. This sensitisation is associated with increased interferon γ production, suggesting a type 1 memory response. We hypothesise that priming of fetal T cells to RSV results in a reduced severity of subsequent RSV disease in these individuals and that this will explain much of the clinical diversity of RSV disease.

Respiratory syncytial virus (RSV) is the predominant cause of viral bronchiolitis (VB), which remains the leading cause for hospitalisation of infants in the developed world.¹ The prevailing opinion is that a combination of declining protection from maternal antibody and small airway size leads to a peak prevalence at 2–4 months of age.² However, these explanations cannot be complete, as all infants have declining maternal antibody concentrations and small airways at this age, but not all develop bronchiolitis.

Cell mediated immunity relies on the clonal expansion of antigen specific T lymphocytes for memory development. It is now clear that antigen specific priming of fetal T cells can occur in utero from 22 weeks gestation.³ We have investigated the possibility of such in utero sensitisation occurring to RSV, and whether this may play a role in the development of VB.

METHODS

Thirty six cord blood specimens were obtained from newborn infants born at the Princess Ann Hospital, Southampton. Cord serum IgA was measured as described by Hide *et al* for all babies.⁴ The A2 strain of RSV was grown in Clone 16 cells (MRC Common Cold Unit, UK) and inactivated by ultraviolet (UV) light exposure. Cord peripheral blood mononuclear cells (PBMC) were separated and resuspended in culture medium with 10% autologous plasma for standard microplate assays, as described previously,³ and cultured for six days in the presence of either uninfected control C16 cells or cells containing UV inactivated RSV (multiplicity of infection (MOI) 1). As a positive control, cells were also cultured for three days with phytohaemagglutinin (PHA, final concentration 1 μ g/ml). DNA synthesis was measured by pulsing with tritiated thymidine for the last 16 hours and counting by liquid scintillation chromatography. A positive proliferative response was defined as delta dpm (counts in stimulated wells – negative controls) >1000 with a stimulation ratio (stimulated well counts/negative controls) >2.

Hospital virological data obtained from the Public Health Laboratory Service diagnostic laboratory at Southampton General Hospital was used to establish the timing of the local RSV epidemic. The epidemic was defined as the date of the first (27 October) and last (10 April) detection of RSV in clinical samples sent in to the laboratory by the hospital or general practitioners in the surrounding area. Using this data the infants were divided into two groups according to whether they were exposed antenatally to the RSV epidemic after 22 weeks gestation (that is, exposed infants were those born between 27 October and 20 August).

The cytokine profile induced by UV inactivated RSV was determined in those subjects exposed in utero to the RSV epidemic after 22 weeks gestation. Aliquots of PBMCs at 1×10^6 /ml were cultured for 24 hours in medium containing either uninfected control C16 cells or cells containing UV inactivated RSV (MOI 1). Supernatant cytokine concentrations were measured using matched antibody pairs optimised for ELISA according to the manufacturers' instructions. Standard curves were based on dilutions of recombinant human cytokines. Interferon γ (IFN γ) (lower detection limit 5 pg/ml), interleukin 10 (IL-10, 6 pg/ml) and IL-12 (6 pg/ml) antibody pairs were obtained from Pharmingen (San Diego, California). IL-4 (0.32 pg/ml) was measured with a high sensitivity ELISA kit commercially available from CLB (Netherlands). Where cytokine concentrations were undetectable, the lower detection limit of the assay was used for statistical analysis.

Data were analysed using χ^2 analysis for categorical data and the Mann–Whitney U test for cytokine differences. Correlations were determined using the non-parametric Spearman test. The study was approved by the Joint Ethics Committee of Southampton University Hospitals.

Abbreviations: IFN, interferon; IL, interleukin; MOI, multiplicity of infection; PBMC, peripheral blood mononuclear cells; PHA, phytohaemagglutinin; RSV, respiratory syncytial virus; UV, ultraviolet; VB, viral bronchiolitis

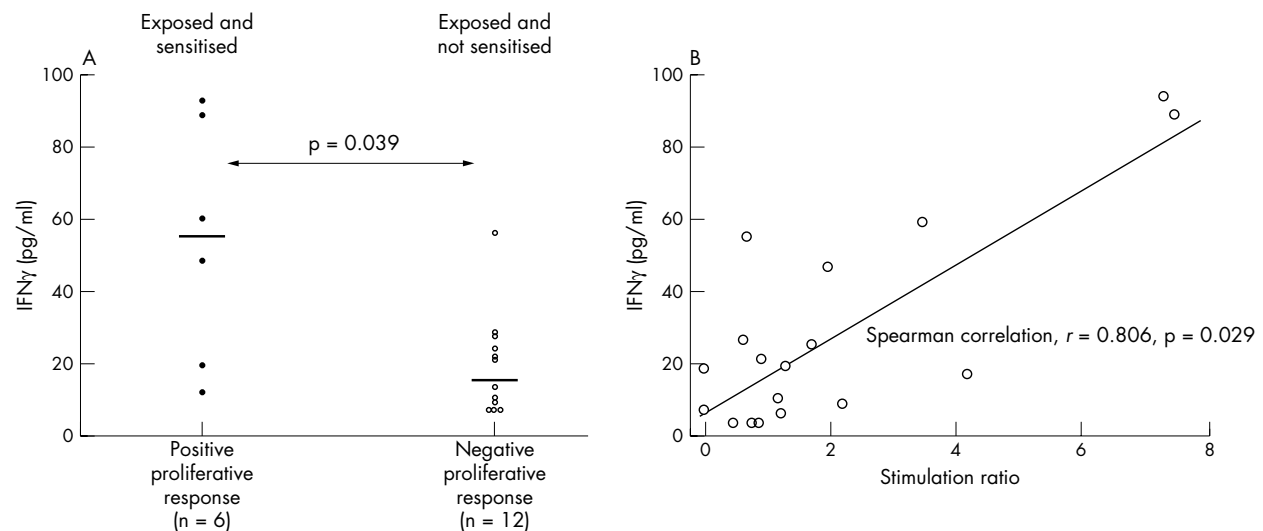


Figure 1 Antenatal sensitisation to RSV is associated with RSV induced IFN γ but not type 2 cytokine production from cord blood mononuclear cells. Cord blood mononuclear cells from the 18 infants whose mothers were potentially exposed to RSV antenatally from 22 weeks gestation were stimulated with UV inactivated RSV. (A) Infants were divided into two groups: sensitised to RSV antenatally (n = 6, filled circles), or not sensitised (n = 12, open circles) according to whether their cord blood mononuclear cells proliferated in response to UV RSV. IL-4 and IL-10 were not produced in response to RSV. Graph shows individual values and median values. RSV induced IFN γ production was significantly augmented in infants with antenatal sensitisation, compared to those not sensitised ($p = 0.039$). (B) A significant correlation was observed between RSV specific proliferation (stimulation index) and IFN γ production ($r = 0.806$, $p = 0.029$).

RESULTS

Total IgA in all cord blood specimens was less than 10 $\mu\text{g/ml}$, indicating an absence of maternal blood contamination.⁵

Infants were divided into two groups according to whether they were exposed antenatally to the RSV epidemic after 22 weeks gestation. Of those not exposed, one of 18 had a positive proliferative response to UV inactivated RSV (i.e. sensitised) compared to six of 18 in the exposed group ($p = 0.035$). PHA stimulation produced a positive proliferative response in all cultures.

IL-4, IL-10, and IL-12 were undetectable in the supernatants of stimulated PBMCs cultured. Following culture with UV inactivated RSV, IFN γ production from specimens with positive proliferative responses (i.e. exposed and sensitised) was significantly higher than from those with negative responses (i.e. exposed but not sensitised) ($p = 0.039$; fig 1A).

The specificity of the PBMC response to stimulation by UV inactivated RSV was shown by the absence of detectable concentrations of IFN γ in supernatants of PBMCs cultured with uninfected C16 cells and the presence of a significant correlation ($r = 0.806$, $p = 0.029$) between IFN γ production and the stimulation ratio (fig 1B).

DISCUSSION

Each year, 1% of all infants are admitted with VB, resulting in a substantial annual health care cost, considerable post-VB morbidity, and occasional deaths.⁶ VB has a peak incidence at 2–4 months of age. Decline in maternal antibody protection and differences in airway calibre can partially explain this age incidence. However, the importance of the host immune response to infection has been increasingly recognised. Animal studies have highlighted the importance of cell mediated immunity in disease pathogenesis with, in particular, an association with a T helper type 2 (Th2) cytokine response in those with a more severe clinical and pathological disease.⁷ Similar immune mechanisms have been implicated in the pathogenesis of human VB.^{8,9} Fetal studies have shown that PBMCs are capable of proliferating in response to mitogenic and allergenic stimuli from around 22 weeks' gestation.³ Further studies have shown that exposure to allergens in utero results in T cell priming—a process that has significant implications for subsequent immunological development.¹⁰

The data presented show that antenatal sensitisation to RSV does occur in one third of infants exposed to a RSV epidemic at the appropriate time of gestation. Furthermore, sensitisation is associated with a type 1 memory response as evidenced by greater IFN γ production in those infants with a positive proliferative response to UV inactivated RSV, compared to those exposed but without such a response. Because of the predictable annual seasonal nature of the epidemic, it is inevitable that those children most at risk will not have had an appropriately timed fetal exposure. Conversely, older infants whose mothers would have been exposed to the virus from 22 weeks' gestation are more likely to have a type 1 memory response. We hypothesise that following such sensitisation, subsequent RSV disease will be of reduced severity in these individuals and this will explain much of the clinical diversity of RSV disease.

Further investigation of this hypothesis will require a large prospective study of a birth cohort followed through the first RSV season. If upheld, this hypothesis would have major implications for therapeutic intervention by suggesting that protection may be obtained by antenatal immunisation of those infants most at risk (with a projected age of 2–4 months in next RSV season).

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