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Immune studies in infants with congenital syphilis

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SUMMARY

Seventeen neonates with congenital syphilis were studied to determine the immune response of the fetus following intra-uterine infection with *Treponema pallidum*. The results were compared with those from healthy controls matched for gestational age, birth weight and sex. B cells, IgM, and circulating immune complexes were significantly elevated in the infected newborns. There were no differences in lymphocyte transformation to phytohaemagglutinin (PHA) and in the CD3, CD4, and CD8 lymphocytes between infants with congenital syphilis and controls. Newborns with congenital syphilis have a heightened humoral response but no quantitative abnormality in cell-mediated immunity. Speculation on the role of the circulating immune complexes is presented.

Keywords congenital syphilis immune response immune complexes

INTRODUCTION

Congenital syphilis continues to be a major clinical problem in the developing world as well as in the industrialized world (Chawla, Gupta & Raghu, 1985; Mascola *et al.*, 1985). Its pathogenesis is not well understood, and the immune response of the affected newborns has not been fully documented.

Most of the research into the immune response in syphilis has been performed in adults with acquired disease (Levene, Wright & Turk, 1971; Jensen & From, 1982; Folds, Maret & Rauchbach, 1982). Reviews on immune mechanisms in syphilis (Musher, Schell & Knox, 1976; Metzger, 1979; Pavia, Folds & Baseman, 1978) have noted that despite the high titres of anti-treponemal IgG and IgM, the humoral immune system on its own neither neutralizes nor confers immunity against the spirochaetal infection. Cell-mediated immune responses were thought to play a major role in protective immunity, a phenomenon emphasized by Friedmann (1977) in one of the few published studies on immune reactivity in congenital syphilis.

Since there are very few published data on the immune response in neonates with congenital syphilis, we examined aspects of the immune response in such infants.

PATIENTS AND METHODS

Patients

Infants in this study were seen in the hospitals of the Peninsula Maternity and Neonatal Service, Cape Town. A diagnosis of early or neonatal congenital syphilis was based on clinical and serological criteria as proposed by Kaufman *et al.* (1977).

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We studied 17 newborns with early congenital syphilis. Their gestational ages as evaluated by the Ballard score (Ballard, Novak & Driver, 1979) ranged from 32 to 37 weeks. Clinical details of the subjects are shown in Table 1. Peripheral blood samples were obtained at the time of clinical presentation, before initiation of treatment.

Affected neonates were matched, on the basis of weight, sex and gestational age, with 17 healthy controls. Informed consent was obtained from all the mothers to enlist their infants in the study and the research protocol was approved by the Ethics and Research Committee of the University of Cape Town.

Lymphocyte transformation assay

Approximately 5 ml of heparinized blood were collected in sterile test tubes. Lymphocyte transformation responses to phytohaemagglutinin (PHA) were measured in microtitre plates as previously described (Beatty & Dowdle, 1979).

T and B cell determinations

$CD3^+$, $CD4^+$ and $CD8^+$ cells were counted using monoclonal antibodies (Orthomune OrthoDiagnostics) (Beatty *et al.*, 1984). B cells were quantified by initially labelling surface membrane immunoglobulins with $F(ab)_2$ rabbit anti-human IgG, IgM and IgA, and then counted with fluorescein-conjugated goat anti-rabbit IgG, IgM and IgA. At least 50 cells were counted. The absolute number of cells was calculated from the total lymphocyte count.

Serum immunoglobulins

Immunoglobulins M, G and A were assayed by turbidimetry (Multistat III Centrifugal Analyzer) in the Chemical Pathology Laboratory, Red Cross Childrens' Hospital, Cape Town. Results were expressed as g/*l*.

Table 1. Clinical details of the patients

	Congenital Syphilis ($n=17$)	Controls ($n=17$)
Birthweight (g)*	2018 ± 581	2008 ± 504
Gestational age (weeks)*	35·4 ± 1·9	35·5 ± 1·6
Sex (M/F)	11/6	11/6
Clinical features		
Hepatomegaly/splenomegaly	15	—
Skin lesions	9	—
Metaphysitis	15	—
Serology		
Elevated VDRL titre	17	—
Positive FTA-IgM$^+$	15	—

* Mean ± s.d.
FTA, Fluorescent treponemal antibody absorption; IgM test.

Serum immune complexes
Immune complexes in serum were assayed by C1q binding and the results expressed as a percentage. (Zubler *et al.*, 1976). In order to obtain normal values for the newborn period serum from cord blood of 20 healthy male and female infants were measured. The assay was performed at the Renal Laboratory, Groote Schuur Hospital, Cape Town.

Haemolytic complement (CH50)
Measurement of the classical complement pathway (CH50) was based on the method of Kabat & Mayer (1971). In one of the patients and in four of the controls serum was insufficient to measure the CH50.

Statistical analysis
Student's *t*-test was employed in the analysis of the data, and $P<0·05$ was taken as significant.

RESULTS

The results of the immune studies are depicted in Table 2. No differences were found in the lymphocyte transformation response to PHA or in the numbers of CD3$^+$, CD4$^+$ or CD8$^+$ cells.

The absolute numbers of B cells were significantly greater in the patients with congenital syphilis. In addition, syphilitic infants displayed significantly elevated levels of serum total IgM. The results of the IgG and IgA are not depicted but there were no differences ($P=0·15$ and $P=0·12$, respectively) between patients and controls.

Measurements of circulating immune complexes (CICs) were significantly raised and CH50 significantly reduced in affected infants. Cord blood immune complexes (ICs) from 20 additional healthy infants had a mean value of 3·1% (s.d. 0·6, range 0–4%).

DISCUSSION

Cell-mediated immune (CMI) responses are now recognized as playing a prime role during active syphilis (Levene *et al.*, 1971; Pavia *et al.*, 1978; Metzger, 1979). Alterations in T cell subsets (Jensen & From, 1982) and reduced reactivity of lymphocytes to mitogens have led researchers to conclude that the CMI response is impaired and blunted in acquired syphilis in adults (Folds *et al.*, 1982; Levene *et al.*, 1969, 1971; Musher, Schell & Knox, 1974).

In this study congenitally infected infants had a normal CMI response as expressed by the absolute numbers of T cells and subsets and the lymphocyte transformation responses to PHA (Table 2). This quantitative response suggests an adequate mobilization of T cells from the lymphoid organs during a syphilitic infection.

However, in the face of an overwhelming infection the lymphoid tissues can become totally depleted of cells leading to a lymphopenia and subsequent death of the infant (Levene *et al.*, 1971). Two neonates in our study died; both had a marked peripheral lymphopenia and died within 24 h of birth.

The lymphocyte transformation assay as a measure of the functional capabilities of lymphocytes has produced inconsistent results in the analysis of CMI responses in adults with syphilis. Some investigators have shown an increase in lymphocyte reactivity (Friedman & Turk, 1975) while others (Levene *et al.*, 1969, 1971; Folds *et al.*, 1982) have concluded that lymphocyte transformation in adult syphilis is impaired. Friedmann (1977) in his study of infant–mother pairs with syphilis showed a reduced activity to treponemal antigen. The different findings from his study and ours could be due to age differences: in the Friedmann study the infants were older (2 months to 2 years) than ours (1–7 days, neonates of various gestational

Table 2. Comparison between infants with congenital syphilis and control infants

Study	Syphilis	*n*	Controls	*n*	*P*
PHA lymphocyte transformation (d/min)	41 523 ± 13 861	17	48 707 ± 12 865	17	0·16
CD3 ($\times 10^9/l$)	3·93 ± 1·37	17	3·16 ± 1·16	17	0·06
CD4 ($\times 10^9/l$)	2·25 ± 1·34	17	1·91 ± 0·77	17	0·74
CD8 ($\times 10^9/l$)	1·17 ± 0·66	17	1·11 ± 0·62	17	0·98
B cells ($\times 10^9/l$)	1·21 ± 0·99	17	0·55 ± 0·28	17	0·003
Total IgM (g/l)	3·79 ± 3·64	17	0·11 ± 0·08	17	0·0007
Immune complexes (% binding)	20·5 ± 18·5	17	3·4 ± 1·9	17	0·007
Haemolytic complement (CH50) (U/ml)	16·7 ± 8·3	16	22·6 ± 6·2	13	0·037

All values expressed as mean ± 1 s.d.

ages); or to the use of the treponemal antigen as the lymphocyte stimulant.

Although human fetal B cells are immature, they are capable of recognizing virtually any antigenic determinant by approximately 24 weeks gestation (Wilson, 1985) and the increased numbers of B cells and elevated IgM levels seen in our syphilitic infants illustrate this (Table 2). Production of a humoral response of such magnitude suggests that there is normal T–B cell interaction. Measurement of serum IgM, either as total IgM on a radioimmunodiffusion plate (Alford, 1971) or as rheumatoid factor in a latex agglutination test (Meyer & Malan, 1987) is an easy and cheap way of screening for congenital syphilis in high risk populations.

This mileu of IgM antibody and presumably antigen excess must inevitably lead to the formation of circulating IgM containing immune complexes and this has been reported by Dobson, Taber & Baughn (1988) in their study of CICs in congenital syphilis. Our study confirms that syphilitic fetuses and newborns have high levels of serum ICs compared with the low levels as measured in cord blood and in the controls. Measurement of the CICs by C1q binding and the low serum CH50 level suggests that the CICs contain antibodies that principally activate the classical complement pathway.

Theofilopoulus & Dixon (1980) in their review of CICs in human disease clearly define the importance of IC formation in the normal immune response. While in most cases this response is beneficial to the host, there are clinical circumstances in syphilitic patients where CICs are associated with organ damage (Wiggelinkhuizen *et al.*, 1973; Jorizza *et al.*, 1986) and to a defective humoral and CMI response (Folds *et al.*, 1982).

Jensen, Jorgensen & Thesturp-Pedersen (1982) in their *in vitro* study on the activity of natural killer cells in syphilitic serum and in the presence of ICs, showed such activity to be suppressed in the presence of both the syphilitic serum and the immune complexes. They concluded that this suppression of natural killer cell activity by ICs provided indirect evidence for the role of ICs as immunosuppressive factors. Pichler, Lum & Broder (1978) in their work on Fc receptors on human T lymphocytes showed that ICs are capable of reacting with suppressor cells, leading to irreversible loss of the Fc receptor for IgG. There are several other studies implicating the role of CICs in defective immune responses (Baughn, Tung & Musher, 1980; Folds *et al.*, 1982). Although the syphiltiic newborn has a normal quantitative immune response as judged by the tests used in our study, the cytotoxic, killing and other functional capabilities of these cells are not known. Such functional abnormalities could account for the clinical impression that infants with congenital syphilis have an increased susceptibility to secondary bacterial infections.

The pathogenesis of the skeletal and placental lesions in congenital syphilis have remained largely unexplained. It is possible that the deposition of CICs in the highly vascularized metaphysis and in the fetal vessels of the placenta are responsible for the pathological lesions. We are at present engaged in such a study.

The immune profile of neonates with congenital syphilis demonstrates a normal CMI response, significantly increased B cells and IgM levels, and an intense production of CICs. Functional abnormalities of T cells have not been excluded and it is postulated that CICs may play a role in the modulation of the immune response and in various tissue lesions.

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REFERENCES

ALFORD, C.A. (1971) Immunoglobulin determinations in the diagnosis of fetal infection. *Pediatr. Clin. N. Amer.* **18,** 99.

BALLARD, J.L., NOVAK, K.K & DRIVER, M. (1979) A simplified score of assessment of fetal maturation of newly born infants. *J. Pediatr.* **95,** 769.

BAUGHN, R., TUNG, K. & MUSHER, D. (1980) Detection of circulating immune complexes in the sera of rabbits with experimental syphilis: possible role in immunoregulation. *Infect. Immun.* **29,** 575.

BEATTY, D.W. & DOWDLE, G.B. (1979) Deficiency in kwashiorkor of serum factors required for optimal lymphocyte transformation *in vitro*. *Clin. exp. Immunol.* **35,** 433.

BEATTY, D.W., HANDZEL, Z.T., PECHT, M., RYDER, C.R., HUGHS, J., MCCABE, K. & TRAININ, N. (1984) A controlled trial of treatment of acquired immunodeficiency in severe measles with thymic humoral factor. *Clin. exp. Immunol.* **56,** 479.

CHAWLA, V., GUPTA, K. & RAGHU, M.B. (1985) Congenital syphilis: a clinical profile. *J. trop. Pediatr.* **31,** 204.

DOBSON, S.R.M., TABER, L.H. & BAUGHN, R.E. (1988) Characterization of the components in circulating immune complexes from infants with congenital syphilis. *J. infect. Dis.* **158,** 940.

ENGEL, S. & DIEZEL, W. (1980) Persistent serum immune complexes in syphilis. *Br. J. vener. Dis.* **56,** 221.

FOLDS, J., MARET, M. & RAUCHBACH, A.S. (1982) Lymphocyte transformation and the effect of circulating immune complexes in humans with syphilis. *Sex. transm. Dis.* **9,** 109.

FRIEDMANN, P.S. & TURK, J.L. (1975) A spectrum of lymphocyte responsiveness in human syphilis. *Clin. exp. Immunol.* **21,** 59.

FRIEDMANN, P.S. (1977) Cell mediated immunological reactivity in neonates and infants with congenital syphilis. *Clin. exp. Immunol.* **30,** 271.

JENSEN, J.R. & FROM, E. (1982) Alterations in T lymphocytes and T-lymphocyte subpopulations in patients with syphilis. *Br. J. vener. Dis.* **58,** 18.

JENSEN, R.J., JORGENSEN, A.S. & THESTRUP-PEDERSEN, K. (1982) Depression of natural killer cell activity of syphilitic serum and immune complexes. *Br. J. vener. Dis.* **58,** 298.

JORIZZA, J.L., MCNEELY, M.C., BAUGHN, R.E., SOLOMON, A.R., CAVALLO, T. & SMITH, E.B. (1986) Role of circulating immune complexes in human secondary syphilis. *J. infect. Dis.* **153,** 1014.

KABAT, E. & MAYER, M. (1971) Complement and complement fixation. In *Experimental Immunochemistry* 2nd edn p. 133. Charles C. Thomas, Springfield, IL.

KAUFMAN, R.E., JONES, O.G., BLOUNT, J.H. & WIESNER, P.J. (1977) Questionnaire survey reported early syphilis: problems in diagnosis, prevention and treatment. *Sex. transm. Dis.* **4,** 135.

LEVENE, G.M., TURK, J.L., WRIGHT, D.J.M. & GRIMBLE, A.G.S. (1969) Reduced lymphocyte transformation due to a plasma factor in patients with active syphilis. *Lancet*, **ii,** 246.

LEVENE, G.M., WRIGHT, D.J.M. & TURK, J.L. (1971) Cell mediated immunity and lymphocyte transformation in syphilis. *Proc. R. Soc. Med.* **64,** 426.

MASCOLA, L., PELOSI, R., BLOUNT, J.H., ALEXANDER, C.E. & CATES, W. (1985) Congenital syphilis revisited. *Am. J. Dis. Child.* **139,** 575.

METZGER, M. (1979) Role of humoral versus cellular mechanisms of resistance in the pathogenesis of syphilis. *Br. J. vener. Dis.* **55,** 94.

MEYER, M.P. & MALAN, A.F. (1987) Rheumatoid factor in the diagnosis of congenital syphilis. *S. Afr. med. J.* **72,** 668.

MUSHER, D.M., SCHELL, R.F. & KNOX, J.M. (1976) Immunologic mechanisms of infectious syphylis. *Cutis*, **17,** 739.

MUSHER, D.M., SCHELL, R.F. & KNOX, J.M. (1974) In vitro lymphocyte response to *Treponema refringens* in human syphilis. *Infect. Immun.* **9,** 654.

PAVIA, C.S., FOLDS, J.D. & BASEMAN, J.B. (1978) Cell mediated immunity during syphilis. A review. *Br. J. vener. Dis.* **54,** 144.

PICHLER, W.J., LUM, L. & BRODER, S. (1978) Fc receptors on human T lymphocytes. I. Transition of Ty to Tu cells. *J. Immunol.* **121,** 1540.

THEOFILOPOULOS, A.N. & DIXON, F.J. (1980) Immune complexes in human disease. A review. *Am. J. Pathol.* **100,** 531.

WILSON, M. (1985) Immunology of the fetus and newborn: lymphocyte phenotype and function. *Clin. Immunol. Allergy*, **5,** 271.

WIGGELINKHUIZEN, J., KASCHULA, R.O.C., UYS, C.J., KUIJTEN, R.H. & DALE, J. (1973) Congenital syphilis and glomerulonephritis with evidence for immune pathogenesis. *Arch. Dis. Child.* **48,** 375.

ZUBLER, R.H., LANGE, G., LAMBERT, P.H. & MIESCHLER, P.A. (1976) Detection of immune complexes in heated sera by a modified ^{125}I-C1q binding test. Effect of heating on the binding of C1q by immune complexes and application of the test to systemic lupus erythematosus. *J. Immunol.* **116,** 232.