Enhanced Anti-Mycobacterial Immunity in Children with Erythema Nodosum and a Positive Tuberculin Skin Test

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Erythema nodosum (EN) may follow a variety of infections, but in regions with a high prevalence of tuberculosis, is frequently associated with a positive tuberculin skin test (TST) and tuberculosis infection. We aimed to investigate the immunological differences between patients with EN as a manifestation of primary tuberculosis, and those with progressive pulmonary tuberculosis (PTB) or asymptomatic infection. We studied the inflammatory response to both mycobacterial and non-mycobacterial antigens in 11 children with EN associated with a positive TST, 22 children with culture-confirmed tuberculosis, and 53 healthy skin test-positive children. In addition, we evaluated functional anti-mycobacterial immunity using an *ex vivo* assay of mycobacterial growth restriction in five children with EN and 15 with PTB. Patients with EN were distinguished by enhanced mycobacterial growth restriction on the functional assay, which was associated with a markedly increased production of IFNγ in response to stimulation with purified protein derivative of *Mycobacterium tuberculosis*. Children presenting with EN and a positive TST show evidence of responses associated with enhanced anti-mycobacterial immunity.

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INTRODUCTION

Erythema nodosum (EN) is an inflammatory condition characterized by the presence of tender subcutaneous nodules distributed symmetrically over the extensor surfaces of the lower extremities. There are well-described associations between EN and a number of underlying disorders, including infections such as tuberculosis, streptococcal pharyngitis, and Epstein-Barr virus as well as non-infectious conditions, such as inflammatory bowel disease, lymphoma, drugs, and sarcoidosis (Cribier *et al.*, 1998). The proportion of cases associated with a particular underlying etiology varies widely, with primary tuberculosis being responsible for a high proportion of cases in regions where tuberculosis is endemic (Kumar and Sandhu, 2004; Mert *et al.*, 2004). The Western Cape region of South Africa has among the highest reported rates of tuberculosis in the world, with an annual incidence of 967/100,000 in 2004 (Tuberculosis Statistics: Cape Town. Western Cape Department of Health. http://www.capegateway.gov.za/Text/2006/5/tb_stats_2006.pdf. Accessed 20 June 2006).

The pathogenesis of EN remains largely unknown. The condition is thought to represent a hypersensitivity reaction to a triggering event. The histological picture of lymphocytes infiltrating the septa between subcutaneous fat nodules may provide an important clue as this pattern resembles a delayed-type hypersensitivity reaction (Fox and Schwartz, 1992). Llorente *et al.* (1997) have previously demonstrated elevated IL-2 and IFNγ gene expression in peripheral blood mononuclear cells and skin biopsies of EN patients, suggesting that a polarized Th1 response occurs in EN.

EN is frequently associated with a positive tuberculin skin test (TST) (Kumar and Sandhu, 2004). As TST reactivity can be due to previous BCG vaccination or exposure to environmental mycobacteria as well as infection with *Mycobacterium tuberculosis*, it is unclear whether the reaction in individual cases of EN reflects a nonspecific heightened responsiveness to mycobacterial antigens shared by *M. tuberculosis*, BCG and environmental mycobacteria, or whether it is the result of a true underlying tuberculosis infection.

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Abbreviations: ELISPOT, enzyme-linked immune spot; EN, erythema nodosum; ESAT-6, expressed secreted antigenic target-6; PPD, purified protein derivative; PTB, pulmonary tuberculosis; TNF, tumor necrosis factor; TST, tuberculin skin test

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The *M. tuberculosis*-specific antigens ESAT-6 and culture filtrate protein 10 have been shown to be useful in distinguishing between tuberculosis infection and prior BCG vaccination, using either *in vitro* enzyme-linked immune spot (ELISPOT) or whole-blood methods to detect T-cell reactivity (Ewer *et al.*, 2003). To address the role of tuberculosis infection in the pathogenesis of EN, and to understand the immunological mechanisms involved, we studied the cytokine responses to both tuberculosis-specific and nonspecific antigens in a group of children with EN and a positive TST, suggesting infection with *M. tuberculosis*. We compared these children with a group of children with active pulmonary tuberculosis (PTB), as well as a group of healthy TST-positive children. In addition, we used a novel whole-blood assay, measuring restriction of mycobacterial growth *in vitro*, to evaluate functional anti-mycobacterial immune responses related to the altered cytokine production.

RESULTS

The characteristics of the children with EN together with their ELISPOT results are given in Table 1. The mean ages of EN cases and healthy controls were similar (99 and 77 months, respectively), but older than the PTB cases (24 months, $P = 0.002$). For the subset of children in whom the whole-blood BCG *lux* assay was performed, the mean age was 48 months in both the PTB and EN groups, but 140 months in the control group. There appeared to be two distinct groups within the EN patients, those presenting at a very young age, and an older group over the age of 8 years. Although the numbers are small, the age distribution differed from patients with PTB (Figure 1). Three of the EN children had abnormal chest radiographs, one showing pneumonic changes together with hilar lymphadenopathy, one a Ghon focus, and one suggestive of hilar lymphadenopathy. All ten of the EN children, those had ELISPOT testing performed, showed ELISPOT responses to purified protein derivative (PPD), in keeping with their positive TST results. However, only seven of these had a positive response to the specific antigen ESAT-6. Of the children in the younger age cluster, only one of the four tested had a positive ESAT-6 response, whereas all of those in the older cluster had a positive response ($P = 0.02$). There was no significant difference in cytokine production between the two age groups.

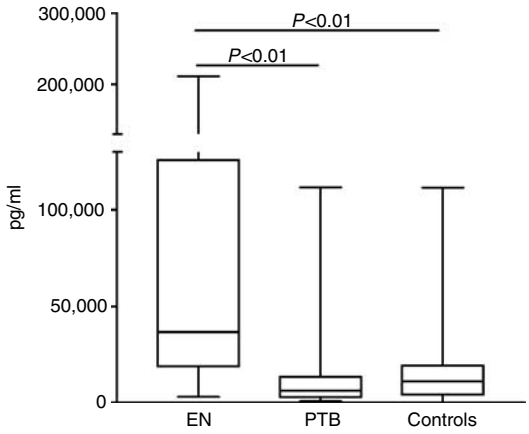

Figure 1. Age distribution of children with EN, children with acute PTB and healthy TST+ children. Lines represent median values. Children with TST+ EN fell into two distinct age groups.

Table 1. Characteristics of subjects with EN and ELISPOT results

Case number	Age (months)	Sex	Chest radiograph	Tuberculin skin test	ELISPOT results (spots per 10^6 lymphocytes)		
					PPD	ESAT-6	Control
EN1	17	F	Normal	Mantoux 15 mm	163	47	2
EN2[1]	19	M	R upper lobe opacification and hilar lymphadenopathy	Mantoux 18 mm	ND[2]	ND	ND
EN3[1]	20	M	Possible hilar lymphadenopathy	Mantoux 20 mm	501	12	2
EN4[1]	21	M	Normal	Tine grade 3	328	0	0
EN5	24	F	Normal	Mantoux 16 mm	216	3	2
EN6[1]	99	F	Normal	Tine grade 4[3]	3,090	113	2
EN7	111	F	Ghon focus[4]	Mantoux 15 mm	385	489	32
EN8	116	F	Normal	Tine grade 4	1,515	112	5
EN9[1]	126	F	Normal	Mantoux 18 mm	810	346	13
EN10	128	F	Normal	Tine grade 4	2,260	147	43
EN11	137	F	Normal	Tine grade 4	59	31	7

[1]BCG *lux* assays performed.
[2]Not done.
[3]Tine test grading: Negative – No induration; Grade 1 – 4-6 papules (also considered negative); Grade 2 – Confluent papules form indurated ring; Grade 3 – Central filling to form disc (positive); Grade 4 – Disc >10 mm with or without blistering (strongly positive).
[4]Pulmonary focus with associated hilar lymphadenopathy.

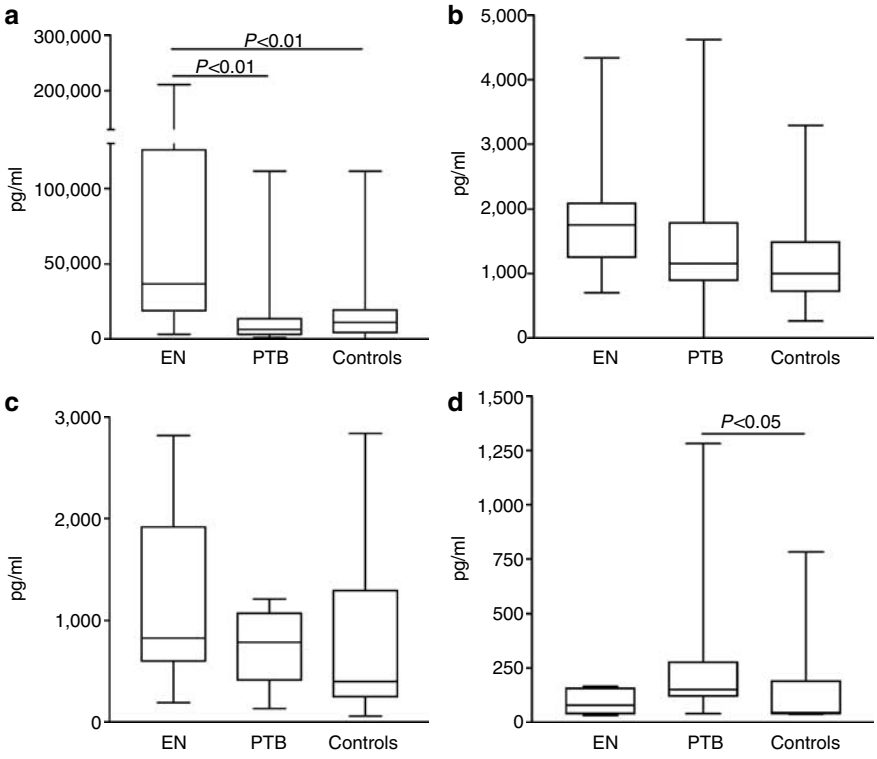

Figure 2. Cytokine responses (a: IFNγ, b: TNF, c: IL12p70, d: IL-10) in whole blood by category. Diluted whole blood was stimulated with PPD (for measurement of IFNγ and IL-10), with LPS (TNF measurement) or LPS plus IFNγ (IL-12p70). Cytokines in culture supernatants were measured by ELISA in children with EN, acute PTB or healthy TST+ children. Plots show median values with quartiles. Whiskers show highest and lowest values. Blood from children with EN produced significantly more IFNγ in response to PPD than healthy TST+ children or children with PTB.

In the whole-blood assay (Figure 2), children with EN produced markedly more IFNγ in response to *M. tuberculosis* PPD, median 36,736 pg/ml) when compared with children with PTB (6,320 pg/ml, $P<0.01$) or healthy controls (11,004 pg/ml, $P=0.002$). Tumor necrosis factor (TNF) responses appeared higher among EN cases (median 1,755 pg/ml) compared with controls (1,002 pg/ml, $P<0.05$) and PTB cases (1,157 pg/ml), but this failed to reach statistical significance ($P=0.069$). IL-12p70 production was similar in EN and PTB cases (median 828 and 786 pg/ml, respectively) and non-significantly lower in controls (400 pg/ml, $P>0.05$). Median levels of IL-10 produced in response to PPD were similar in the EN (78 pg/ml) and healthy children (40 pg/ml). Children with active PTB tended to produce more IL-10 (median 151 pg/ml, $P=0.0194$).

The growth of BCG *lux* was measured *in vitro* in five children with EN and compared with 15 children with PTB and eight healthy control children with TST >15 mm (Figure 3). A growth ratio (GR) was calculated from this assay, which reflects the ability of whole blood to restrict growth of BCG. A low GR implies greater growth restriction. Blood from children with EN (median GR = 1.5) restricted growth of BCG *in vitro* better than blood from children with PTB (median GR = 5) or healthy TST+ children (GR = 6, $P=0.01$). Previous results from our laboratory confirmed that there is no age dependency in growth restriction (data not shown).

DISCUSSION

EN is associated with a strong IFNγ response and restriction of mycobacterial growth *ex vivo*

Studies in both mice and humans have identified the Th1 axis, with IFNγ as a central player, as an important role in protection against mycobacterial infection (Sullivan *et al.*, 2005). Individuals with genetic defects in Th1 cytokine production or response elements are predisposed to severe infections with both non-tuberculous mycobacteria and tuberculosis (Newport and Levin, 1999).

It is therefore of interest that blood from children with EN produced markedly more IFNγ in response to stimulation with PPD when compared with either healthy TST+ children or children with culture-confirmed PTB. Children with active PTB tended to produce the lowest levels of IFNγ as expected, given the poor antigen-specific responses previously documented among children with active infection (Ellner, 1997). A further possible explanation for the lower levels of IFNγ in this group is their younger age, given the reduced functional capacity of T lymphocytes in early life (Wilson *et al.*, 1992). There was a trend towards increased production of another Th1 cytokine, TNF, in response to LPS stimulation among EN patients. In contrast, PPD-stimulated levels of the regulatory cytokine IL-10 were lower in EN patients and controls than in PTB cases. This pattern of reactivity, with high levels of the proinflammatory cytokines IFNγ, and TNF, and low levels of the regulatory cytokine IL-10, suggests that EN is associated

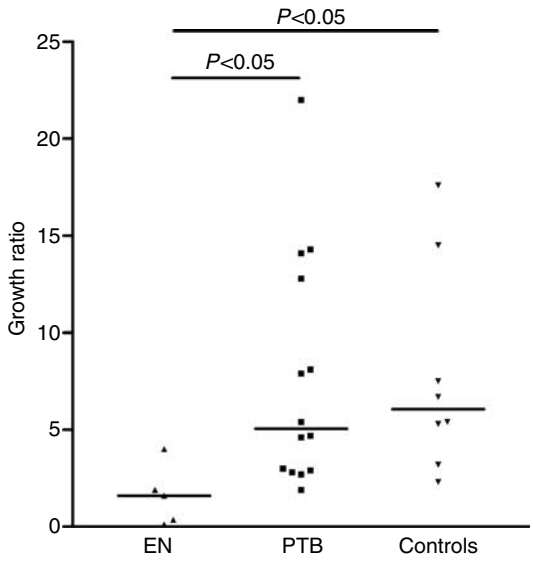

Figure 3. BCG *lux* GRs by category. Diluted whole blood was incubated with BCG *lux* for 96 hours and the GR calculated using the formula: luminescence at 96 hours/luminescence at 0 hour. Children with EN, acute PTB and healthy TST + children. Lines represent median values. Blood from children with EN restricted the growth of BCG *lux* significantly more than healthy TST + children or children with PTB.

with a more intense pro-inflammatory response than is seen in PTB.

The second distinctive finding in the children with EN, as compared with those with PTB or healthy controls, was the significantly enhanced functional ability of blood to restrict the growth of mycobacteria *ex vivo*. We have previously used this assay to show that BCG vaccination enhances mycobacterial growth restriction (Kampmann *et al.*, 2004) and that growth restriction improves as immune reconstitution occurs during highly active antiretroviral therapy (Kampmann *et al.*, 2006). This assay is therefore a useful marker of anti-mycobacterial immunity and our results suggest that the EN patients are more able to restrict the growth of mycobacteria than PTB patients or healthy controls. The control children for this assay were significantly older than the EN patients. However, this is unlikely to confound these results, as age does not significantly affect growth restriction among BCG-vaccinated children (B Kampmann, unpublished data) (in any event, the trend might be towards underestimating the magnitude of any difference, as older children are assumed to have stronger anti-mycobacterial immunity).

As IFNγ and TNF are key cytokines involved in the activation of macrophages to enhance mycobacterial killing, these data, together with the increased functional ability to restrict mycobacterial growth, suggests that EN occurs in children with strong anti-mycobacterial immunity. These enhanced responses are apparent in peripheral blood. Further work examining the precise nature of the immune response at the site of the lesions is required to relate the circulating responses to the skin manifestations of EN.

It is interesting to draw a parallel with EN leprosum, an immunologically mediated reaction to a different mycobacterium, *Mycobacterium leprae*. EN leprosum is also characterized by subcutaneous nodules on the lower extremities as well as by arthralgias and fever. EN leprosum is characterized by a Th1 cytokine profile with detectable IFNγ and IL12p40 RNA in unstimulated circulating peripheral blood mononuclear cells (Sreenivasan *et al.*, 1998). Further, the treatment of multibacillary leprosy with recombinant human IFNγ is associated with the development of EN leprosum (Sampaio *et al.*, 1996). In both conditions, therefore, the presence of high levels of IFNγ (whether endogenous or exogenous) appears central to the pathogenesis of the condition.

Older children with EN had positive responses to the tuberculosis-specific antigen, ESAT-6

The nature of the antigenic stimulus for EN varies according to geographic location. In South Africa, where tuberculosis is highly prevalent, tuberculosis is likely to be an important triggering event. However, BCG vaccine coverage at birth is in excess of 95% in South Africa (Saloojee and Bamford, 2006). All cases of EN reported completion of vaccination, however, vaccine records were not available for review in the majority of cases.

It is therefore feasible that the positive TST documented in many cases of EN could be associated with a nonspecific polarized Th1 response to antigens such as BCG or environmental mycobacteria.

We sought to test this hypothesis by testing ELISPOT responses to the tuberculosis-specific antigen ESAT-6. Cases with EN fell into two discrete age clusters with median ages of 20 and 119 months. Interestingly, younger children presenting with EN were unlikely to have a positive response to ESAT-6. We and others (Nicol *et al.*, 2005) have previously shown that over 80% of children with proven active tuberculosis have positive ELISPOT responses to tuberculosis-specific antigens. It is possible that the positive TST in this group of young children may be associated with recent BCG vaccination, rather than with infection with *M. tuberculosis*. An alternative explanation is that the development of specific IFNγ responses is delayed in the younger children. Several studies indicate an age-related increase in IFNγ responses (Hartel *et al.*, 2005), and the lack of ELISPOT response thus may reflect this age-related delay in maturation. However, we believe that this is an unlikely explanation, given the strong ELISPOT responses to PPD but not ESAT-6 in these children, suggesting that EN in these cases was unlikely to have been due to recent tuberculosis infection.

In contrast, all six children with EN in the older group had a positive response to ESAT-6, suggesting that the precipitating stimulus in these cases was recent infection with *M. tuberculosis*. Currently, most tuberculosis-specific ELISPOT assays employ two specific antigens, ESAT-6 and culture filtrate protein 10. One drawback of this study was that only ESAT-6 was used for testing. It is feasible that additional cases would have responded to culture filtrate protein 10, had this antigen been available. Furthermore, repeat of the ELISPOT after a delay of 1–2 months, may have helped to establish whether the specific response was delayed rather than

absent. *In vitro* IFNγ-release assays that are able to discriminate between prior BCG vaccination and tuberculosis infection may, therefore, play an adjunctive role in the clinical evaluation of children with a positive TST and EN.

When a child presents with EN, a positive TST and no other clinical, radiological, or microbiological evidence of tuberculosis, the differentiation between tuberculosis infection and active disease is unclear. The presence of EN and a positive TST always requires a careful clinical examination and chest radiograph to determine whether there is underlying active disease.

In summary, blood from children with EN and a positive TST shows an enhanced ability to restrict growth of mycobacteria *ex vivo* and marked production of IFNγ in response to PPD when compared to both children with active PTB as well as healthy skin test-positive children. These are features associated with strong anti-mycobacterial immunity. Older children with EN were more likely to have positive responses to the tuberculosis-specific antigen ESAT-6, suggesting recent tuberculosis infection.

MATERIALS AND METHODS

Subjects

Eleven children (<14 years of age) presenting to the dermatology outpatient clinic with EN and positive TST were investigated. They were compared with children with newly diagnosed PTB within the first 3 weeks of therapy and with 53 healthy children with a TST (Mantoux) skin test of ⩾15 mm recruited from the same community. Two groups of children with PTB were recruited. The first PTB group of 22 children with culture-confirmed tuberculosis was used for comparison of cytokine responses using ELISPOT and stimulated whole-blood culture supernatants. A second group of 15 children with PTB was enrolled for comparison of mycobacterial growth in whole blood, as the volumes of blood required precluded undertaking both assays on the same patients. TST-positive healthy children were recruited by visiting homes of adults with previously diagnosed smear-positive tuberculosis. The decision to place a child on anti-tuberculous therapy was made by the attending clinician and was not influenced by the study results. All healthy controls with positive TST were referred to their local clinic for investigation and management according to the National Tuberculosis Control Programme guidelines. Following the consent procedure, 5–10 ml of blood was collected from each child by peripheral venipuncture. Approval to undertake the study was obtained from the Research Ethics Committee of the University of Cape Town and informed consent obtained from a parent or main caregiver before inclusion in the study. The study was conducted according to the Declaration of Helsinki Principles.

Among the children with EN, whole-blood cytokine assays were performed in all cases. Sufficient blood was available for ELISPOT assays in ten of the 11 children. In five of the children with EN, adequate blood was available to perform BCG *lux* growth assays.

TST of controls was performed by a study nurse, using two tuberculin units of PPD RT23 (Statens Serum Institute, Copenhagen, Denmark) and induration diameters measured 48–72 hours later. TST of children with EN (Mantoux or tine testing) was performed by the attending clinician (the TST method varied with the hospital where the children were recruited).

Whole-blood assay for TNF, IL-12p70 IL-10 and IFNγ measurements

We used a range of assays designed to probe the integrity of the IFN-γ/IL-12 response pathway by measuring the production of key cytokines important in the inflammatory response to mycobacteria. Whole blood, collected into preservative-free heparin, was diluted 1:10 in RPMI supplemented with penicillin and streptomycin plus 2 mM L-glutamine (Gibco BRL, Paisley, Scotland) and 180 μl pipetted into 96-well, round-bottomed tissue culture plates (Nunc, Roskilde, Denmark). For each condition, four wells were used and supernatants pooled. For measurement of IL-12p70, 10 μl of rHu IFN-γ (final concentration 1 μg/ml) was added, incubated at 37°C in 5% CO_2 for 2 hours after which 20 μl of LPS from *Escherichia coli* 0111:B4 (final concentration 10 ng/ml) was added. Incubation was continued for a further 18 hours at 37°C in 5% CO_2. For measurement of TNF, LPS (final concentration 0.5 ng/ml) was used and supernatant harvested after 18 hours. For IFNγ and IL-10 measurement, PPD (Evans Pharmaceutical, Weybridge, Surrey, UK) at a final concentration of 10 μg/ml was used as a stimulant and incubated for 6 days. All supernatants were stored at −80°C.

IFN-γ, IL-10, TNF, and IL-12p70 were measured by ELISA using antibody pairs purchased from BD Pharmingen. Results are expressed as the mean of duplicate readings for each sample, with a coefficient of variation of less than 15%.

ELISPOT assays

The number of T cells specifically reacting to the tuberculosis-specific antigen ESAT-6 or the less specific antigens in PPD were evaluated using the ELISPOT assay performed as described previously (Lalvani *et al.*, 2001). Briefly, peripheral blood mononuclear cells were separated by Ficoll-Paque centrifugation. Cells were washed, re-suspended, and counted.

PVDF-backed plates (96-well) (MAIPS4510, Millipore, Bedford, MA) were coated with 15 μg/ml of anti-IFN-γ mAb 1-D1K (Mabtech, Nacka, Sweden). Cells (300,000 or 100,000 per well) were added to duplicate wells containing 5 μg/ml ESAT-6 (Statens Serum Institut, Copenhagen), 10 μg/ml PPD (Evans Vaccines, UK) or mitogen (PHA, 5 μg/ml). No antigen was added to the background control wells. After 18 hours incubation, plates were washed. Biotinylated anti-IFN-γ mAb (100 μl of 1 μg/ml), 7-B6-1-biotin (Mabtech), was added for 2 hours. Plates were then washed and streptavidin-alkaline phosphatase toxoid (Mabtech) was added. After 1.5 hours and further washing, 100 μl of chromogenic alkaline phosphatase substrate (Biorad, Hercules, CA) was added. After 10–15 minutes, plates were washed and spots enumerated independently by two observers using a stereomicroscope. The mean values of the two observers (mean inter-observer coefficient of variation = 8%) and both duplicate wells are used in all calculations. Observers were blinded to the clinical status of the patient.

Whole-blood BCG lux assay

The functional ability to restrict the growth of mycobacteria was evaluated using the whole-blood luciferase-tagged BCG (BCG *lux*) assay as previously described (Kampmann *et al.*, 2004). Briefly, triplicate samples of heparinized venous blood, diluted with an equal volume of RPMI were infected with 1×10^7 relative light units

(RLU)/ml (equal to 1×10^6 colony-forming units (CFU)/ml) of BCG *lux* and incubated for 96 h with continuous mixing of the samples. Following lysis of red blood cells, mycobacterial viability was measured at time of inoculation (T_0) and at 96 h (T_{96}) using a luminometer (Berthold). The GR was calculated using the formula GR = RLU of BCG *lux* at T_{96}/RLU of BCG *lux* at T_0.

Statistical analysis

A response which was >30 spots per million peripheral blood mononuclear cells and greater than twice the response in the negative control wells was regarded as positive for ELISPOT analysis. For comparison of ages and cytokine responses in each group the Kruskal–Wallis test was used together with Dunn's post test. Fisher's exact test was used for contingency analysis of the number of positive ELISPOT responses in each age category. For comparison of GRs the Mann–Whitney test was used. A *P*-value of less than 0.05 was regarded as significant for all tests.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

Cribier B, Caille A, Heid E, Grosshans E (1998) Erythema nodosum and associated diseases. A study of 129 cases. *Int J Dermatol* 37:667–72

Ellner JJ (1997) Regulation of the human immune response during tuberculosis. *J Lab Clin Med* 130:469–75

Ewer K, Deeks J, Alvarez L, Bryant G, Waller S, Andersen P *et al.* (2003) Comparison of T-cell-based assay with tuberculin skin test for diagnosis of *Mycobacterium tuberculosis* infection in a school tuberculosis outbreak. *Lancet* 361:1168–73

Fox MD, Schwartz RA (1992) Erythema nodosum. *Am Fam Physician* 46:818–22

Hartel C, Adam N, Strunk T, Temming P, Muller-Steinhardt M, Schultz C (2005) Cytokine responses correlate differentially with age in infancy and early childhood. *Clin Exp Immunol* 142:446–53

Kampmann B, Tena GN, Mzazi S, Eley B, Young DB, Levin M (2004) Novel human *in vitro* system for evaluating antimycobacterial vaccines. *Infect Immun* 72:6401–7

Kampmann B, Tena-Coki GN, Nicol MP, Levin M, Eley B (2006) Reconstitution of antimycobacterial immune responses in HIV-infected children receiving HAART. *AIDS* 20:1011–8

Kumar B, Sandhu K (2004) Erythema nodosum and antitubercular therapy. *J Dermatolog Treat* 15:218–21

Lalvani A, Pathan AA, McShane H, Wilkinson RJ, Latif M, Conlon CP *et al.* (2001) Rapid detection of *Mycobacterium tuberculosis* infection by enumeration of antigen-specific T cells. *Am J Respir Crit Care Med* 163:824–8

Llorente L, Richaud-Patin Y, Alvarado C, Reyes E, Cocer-Varela J, Orozco-Topete R (1997) Elevated Th1 cytokine mRNA in skin biopsies and peripheral circulation in patients with erythema nodosum. *Eur Cytokine Netw* 8:67–71

Mert A, Ozaras R, Tabak F, Ozturk R (2004) Primary tuberculosis cases presenting with erythema nodosum. *J Dermatol* 31:66–8

Newport M, Levin M (1999) Genetic susceptibility to tuberculosis. *J Infect* 39:117–21

Nicol MP, Pienaar D, Wood K, Eley B, Wilkinson RJ, Henderson H *et al.* (2005) Enzyme-linked immunospot assay responses to early secretory antigenic target 6, culture filtrate protein 10, and purified protein derivative among children with tuberculosis: implications for diagnosis and monitoring of therapy. *Clin Infect Dis* 40:1301–8

Saloojee H, Bamford L (2006) Key childhood health promotion and disease prevention programmes. In: *South African Health Review 2006* (Ijumba P, Padarath A, eds), Durban: Health Systems Trust, 181–202

Sampaio EP, Malta AM, Sarno EN, Kaplan G (1996) Effect of rhuIFN-gamma treatment in multibacillary leprosy patients. *Int J Lepr Other Mycobact Dis* 64:268–73

Sreenivasan P, Misra RS, Wilfred D, Nath I (1998) Lepromatous leprosy patients show T helper 1-like cytokine profile with differential expression of interleukin-10 during type 1 and 2 reactions. *Immunology* 95:529–36

Sullivan BM, Jobe O, Lazarevic V, Vasquez K, Bronson R, Glimcher LH *et al.* (2005) Increased susceptibility of mice lacking T-bet to infection with *Mycobacterium tuberculosis* correlates with increased IL-10 and decreased IFN-gamma production. *J Immunol* 175:4593–602

Wilson CB, Penix L, Weaver WM, Melvin A, Lewis DB (1992) Ontogeny of T lymphocyte function in the neonate. *Am J Reprod Immunol* 28:132–5