Abstract. A 4-year-old girl presented with recurrent infections. Immunoglobulin deficiency (serum and secretory IgA, serum IgG3) neutropenia and neutrophil dysfunction (defective spontaneous migration and chemotaxis) were found. T-lymphocyte counts were normal and they responded to phytohaemagglutinin but were not stimulated by Concanavalin A, pokeweed mitogen and microbial antigens in vitro. Delayed cutaneous hypersensitivity testing to purified protein derivative and candidin was negative. Despite bacille Calmette-Guérin vaccination and candidiasis, near normal [3-2-microglobulin and human leucocyte antigen (HLA) class I concentrations were detected on mononuclear cells and phytohaemagglutinin-induced lymphoblasts. HLA class II antigens (HLA-DP, -DQ, -DR) were not expressed. These observations indicated a bare lymphocyte syndrome (BLS) type II. This is the first time neutrophil dysfunction has been noted in association with BLS.

Key words: Bare lymphocyte syndrome – HLA expression – Severe combined immunodeficiency syndrome – Neutrophil dysfunction

Introduction

Bare lymphocyte syndrome (BLS) was first described by Touraine et al. in 1978 [20]. This combined immunodeficiency syndrome is characterised by recurrent severe infections of the respiratory and gastro-intestinal tracts [7] and is associated with a lack of expression of human leucocyte antigen (HLA) antigens on mononuclear cells [20].

We present a patient with recurrent pneumonia and parotitis, salmonella enteritis, intestinal and genital candidiasis refractory to treatment. In addition to defective expression of HLA antigens, our patient showed partial neutrophil dysfunction.

Case report

The patient was born in the 35th week of pregnancy as the fourth child of healthy consanguineous Tunisian parents (5th cousins). The second child was stillborn; post mortem examination revealed interstitial myocarditis. The other sibs are healthy.

Postpartal bacille Calmette-Guérin (BCG) vaccination resulted in uncomplicated scar formation. Within 8 months she had two bouts of pneumonia, one episode of gastro-enteritis and one oro-genital candida infection.

On admission to hospital at 9 months because of enteritis, the patient weighed 4675 g (under 3rd percentile) and measured 61 cm (under 3rd percentile). Stool cultures revealed Salmonella wien. Laboratory analysis showed leucocytosis (15100 cells/gl) with relative neutropenia (13%) and relative lymphocytosis (81%). Follow-up revealed absolute neutropenia (500-1000 cells/gl) with normal leucocyte count. Serum IgG was 2.5 g/l (normal 3.4-10.8 g/l), IgM 0.271 g/l (normal 0.36-1.84 g/l) IgA 0.19 g/l (normal 0.16-0.90 g/l).

At 18 months the patient was re-admitted because of parotitis. No pathogens were detected by culture or serology. Hypogammaglobulinaemia persisted and immunoglobulins were administered every 3 weeks (7-s-IgG 0.4 g/kg body weight i.v.).

Three months later the parotitis recurred, accompanied by tonsillitis, severe salmonella enteritis and intestinal candidiasis. Antibiotics, antifungal drugs and i.v. rehydration were given in addition to immunoglobulins. Because of the severe secretory IgA deficiency (see Table 2), IgA was given orally on an experimental basis (460 mg/kg body weight for 28 days; Igabolin, Immuno, Heidelberg, FRG).

Because of these clinical and laboratory findings the possibility of severe combined immunodeficiency was elucidated, and BLS finally diagnosed (see Table 1).
Appropriate treatment was administered; e.g., cotrimoxazole, ketonazole/miconazole as well as regular immunoglobulin substitutions. This supportive therapy was continued until at the age of 28 months when bone marrow transplantation (BMT) was performed at the Hôpital des Enfants Malades, Paris, using a haploidentical bone marrow harvested from the father. Thirty months post-transplant, the patient is in good general health.

Materials and methods

All immunological investigations were performed when the patient was aged 1 year and 10 months.

Cell separation

A suspension of neutrophils (PMN) and peripheral blood mononuclear cells (PBMC) containing monocytes (MN) and peripheral blood lymphocytes (PBL) was prepared from heparinised peripheral blood by sedimentation with Dextran and/or Ficoll-Hypaque/Percol centrifugation. The PMN preparation consisted of 95% neutrophils. The PBMC preparation contained 70%–90% PBL, 10%–15% MN and 10% PMN.

Monoclonal antibodies (MAb)

MAb that recognize β2-microglobulin (a), HLA-ABC (b), HLA- DP (c), -DQ (d) and -DR (e) were purchased from Becton-Dickinson, Basle, Switzerland (a, c, d, e), from Ortho Pharmaceutical, Ravitan NJ, USA (e), from Dako, Zurich, Switzerland (e) and from Immunotech, Marseilles, France (b).

Immunofluorescence studies

Indirect immunofluorescence of intact cells was carried out using the MAb and fluorescein-conjugated goat anti-mouse immunoglobulin (New England Nuclear, Regensdorf, Switzerland). Preparations were evaluated using an epilumination microscope. The sheep F(ab')2 anti-mouse immunoglobulin alone labelled cells specifically.

Flow cytometric analysis

This was carried out using the MAb and fluorescein-conjugated goat anti-mouse Ig (F(ab')2) from Ortho, Spireitenbach, Switzerland. The cells were analysed in a Becton Dickinson FAC scan (Becton Dickinson, Basle, Switzerland). The cutoff for positive cells was set according to the fluorescence of the control antibody and the percentage of each population was calculated accordingly.

Immunoglobulins and antibodies

Serum immunoglobulin levels were measured 2 days after i.v. IgG infusion by nephelometry (IgG, IgA, IgM) or by radial immunodiffusion (IgG1, IgG2, IgG3, IgG4). Secretory IgA and secretory component were measured by Rocket immunoelectrophoresis. Serum antibody concentrations were determined by haemagglutination (anti-B).

Lymphocyte markers and stimulations

B- and T-lymphocytes (CD2, CD4 and CD8 positive lymphocytes) were enumerated by immunofluorescence using specific monoclonal antibodies (Becton Dickinson, Basle, Switzerland). Mitogen (phytohaemagglutinin [PHA], concanavalin A [Con-A], pokeweed mitogen [PWM] and antigen (purified protein derivative [PPD], candidin) induced lymphocyte proliferations were measured using ³H-thymidine incorporation. Skin tests were performed with PPD and candidin using a commercial applicator (Bio Mérieux, Lyon, France).

Chemotaxis

Chemotaxis was studied by a modified underagarose technique [9]. The chemo-attractant used was zymosan-activated normal serum. Incubation was performed in a 5% CO₂ atmosphere at 37°C for 120 min.

Phagocytosis

Phagocytic assay mixtures contained 10⁶ PMN, 2.5 × 10⁷ yeast particles, and 10% pooled normal serum. Incubation was carried out at 37°C for 30 min.

Measurement of chemiluminescence

Production of chemiluminescence from PMN was measured after stimulation with opsonized zymosan (1 mg/ml) using a previously described luminol-dependent photometric assay [10].

Conventional HLA-typing and restriction fragment length polymorphism (RFLP) technique

PBMC were isolated from heparinised blood by density flotation using a Ficoll gradient (Pharmacia, Uppsala, Sweden). Cells were further separated into non-adherent (T-cells) and adherent (B-cells/monocytes) populations by passage through a nylon wool column. HLA class I typing was performed on non-adherent cells and HLA class II on adherent cells by the National Institutes of Health microlymphocytotoxicity test [1].

HLA class II typing was also performed by the RFLP technique according to a modified procedure based on that described by Font et al. [3]. Briefly, DNA was extracted from the granulocyte pellets (Ficoll gradients), digested with the restriction enzymes EcoRI, TaqI, HindIII, BamHI, PstI (6 units/µg; Boehringer, Mannheim, FRG), and electrophoresed in 0.7% agarose

Table I. Cell surface HLA-antigen expression on mononuclear cells and PHA-induced lymphoblasts

<table>
<thead>
<tr>
<th></th>
<th>Patient</th>
<th>Control (adult)</th>
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<tbody>
<tr>
<td>Mononuclear cells⁷</td>
<td>84</td>
<td>96</td>
</tr>
<tr>
<td>β-2-Microglobulin (%)</td>
<td>75</td>
<td>96</td>
</tr>
<tr>
<td>HLA class I (%)</td>
<td>2</td>
<td>51</td>
</tr>
<tr>
<td>HLA-DP (%)</td>
<td>1</td>
<td>30</td>
</tr>
<tr>
<td>HLA-DQ (%)</td>
<td>3</td>
<td>40</td>
</tr>
<tr>
<td>HLA-DR b (%)</td>
<td>1 1</td>
<td>23</td>
</tr>
<tr>
<td>PHA-induced lymphoblasts⁷</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-2-Microglobulin (%)</td>
<td>93</td>
<td>92</td>
</tr>
<tr>
<td>HLA class I (%)</td>
<td>98</td>
<td>94</td>
</tr>
<tr>
<td>HLA-DQ (%)</td>
<td>8 4</td>
<td>46</td>
</tr>
<tr>
<td>HLA-DR b (%)</td>
<td>9 5</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>2 5</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>8 4</td>
<td>41</td>
</tr>
</tbody>
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⁷ Analysis performed by flow cytometry (see Fig. 2)

b Determined with 3 different monoclonal antibodies (I, II, III) for anti-HLA-DR