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Defective neutrophil and lymphocyte function in leucocyte adhesion deficiency

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SUMMARY

We report a Chinese girl with the moderate phenotype of leucocyte adhesion deficiency (LAD), presenting with persistent omphalitis and recurrent soft tissue infections. She had subnormal adhesion-dependent neutrophil functions, such as chemotaxis and chemiluminescence response to a particulate stimulant (opsonised zymosan). Despite her adequate humoral response to documented herpes simplex virus type 1, parainfluenza type 2 and adenovirus infection *in vivo*, there was marked impairment in the generation of plaque-forming cells (PFC) driven by pokeweed mitogen (PWM) *in vitro*. IgM PFC were less severely affected than IgG and IgA PFC, probably because IgM production is less dependent on T cell help than IgA and IgG production. The patient's B cells and accessory cells had reduced function compared with the control subsets, while helper function of her $CD4^+$ cells was virtually absent in the PWM-driven PFC assay. She also had marked defect in natural killer cell activity. The proliferation of her lymphocytes was normal to several plant lectins, including phytohaemagglutinin, concanavalin A and PWM, but markedly defective to OKT3.

Keywords LFA-1 Mac-1 plaque-forming cells memory T cells Chinese

INTRODUCTION

Leucocyte adhesion deficiency (LAD) is an autosomal recessive disease due to mutations in the gene encoding the common β subunit of the three leucocyte surface heterodimers, i.e lymphocyte function-associated antigen-1 (LFA-1), complement receptor type 3 (Mac-1) and p150,95 (Kishimoto *et al.*, 1987a, 1987b). This results in either absent or low expression of the three heterodimers, causing severe impairment of adhesion-dependent leucocyte cell functions, resulting in a syndrome characterized by delayed umbilical cord separation, recurrent soft-tissue infections, diminished pus formation, granulocytosis, poor wound healing and progressive periodontitis (Anderson *et al.*, 1985; Anderson, 1987). This syndrome has been described in Caucasian, Iranian, Hispanic and Japanese patients (Abramson *et al.*, 1981; Bowen *et al.*, 1982; Arnaout *et al.*, 1982, 1984; Anderson *et al.*, 1984; Beatty *et al.*, 1984; Dana *et al.*, 1984; Kobayashi *et al.*, 1984; Fischer *et al.*, 1985). We report here the first case in Chinese with the moderate phenotype of leucocyte adhesion deficiency, exhibiting not only neutrophil and natural killer (NK) cell dysfunction but also defective interactions between helper T cells, B cells and accessory cells.

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CASE REPORT

The patient is the younger of two children of consanguinous parents. Her paternal grandfather and maternal grandmother were full siblings; the paternal grandmother and maternal grandfather were half-siblings. Her umbilical stump separated normally but persistent omphalitis developed. Umbilical swabs grew *Staphylococcus aureus*, *Bacteroides fragilis* and *Streptococcus faecalis* at different times. Clinical response to antibiotics was poor. A urachal cyst was excised at 3 months of age. The patient had persistently elevated leucocyte counts with a predominance of neutrophils, the highest being $133 \times 10^9/l$. At 5 months, she was re-admitted with right temporal abscess and *S. aureus* was isolated. She developed two more abscesses despite appropriate antibiotics. She was suspected to have LAD, and this was confirmed by subsequent investigations.

At 7 months, the patient was admitted with protracted diarrhoea and failure to thrive. She developed perianal cellulitis, from which *Pseudomonas aeruginosa* was grown. She had a four-fold increase of titre against adenovirus during this admission, but no virus was ever isolated from the stool. She also contracted nosocomial bronchiolitis due to respiratory syncytial virus (RSV). She recovered without specific treatment. At 10 months, she was admitted with fever and coryzal symptoms. Her paired sera showed an eight-fold increase of titre against parainfluenza type 2 virus. She again recovered with no complication.

The patient was admitted at 12 months of age with perianal abscess and gingivostomatitis. Several skin vesicles appeared on her left foot. Herpes virus was demonstrated on electron microscopy of the vesicular fluid. Herpes simplex virus (HSV) type 1 was isolated by culture. Multiple sera showed an increase in titre against HSV from < 1 in 10 to 1 in 40 over 4 weeks. She was given acyclovir but there was no evidence of disseminated infection. She developed cellulitis around the left angle of her mouth, which healed with scarring. She had recurrent HSV infection of the left angle of her mouth at 14 months of age and had a further increase in titre against HSV from 1/40 to 1/160. She was recently tested for delayed-type skin hypersensitivity using intradermal injection of 0·1 ml of Candida extract, Tetanus toxoid, 10 U of purified protein derivative and 5 U of streptokinase. She had a strong positive reaction to Tetanus toxoid (20-mm induration) and was negative to the other three antigens. The patient is 2·5 years old now, and is still being admitted regularly for soft tissue abscesses.

MATERIALS AND METHODS

Serology

Immunoglobulin levels were determined by rate nephelometry using the Cobas Bio fast centrifugal analyser (Roche, Basle, Switzerland).

Cell separation

Leucocytes were obtained by standard methods of dextran sedimentation or Ficoll–Hypaque centrifugation of heparinized blood. Cells were washed three times in HBSS and adjusted to the appropriate concentration.

Monoclonal antibodies (MoAbs)

OKT3 (mouse IgG2a, anti-CD3), OKT4 (mouse IgG2b, anti-CD4), OKT8 (mouse IgG2, anti-CD8) and OKM1 (mouse IgG2b, anti-CD11b) were obtained from Ortho (High Wycombe, UK); Leu 11b (mouse IgM, anti-CD16) was from Becton Dickinson (Mountain View, CA); anti-CD11a (mouse IgG2) and anti-CD18 (mouse IgG1) were obtained from Janssen (Beerse, Belgium). Immunomagnetic beads coated with MoAbs against CD4, CD8 and CD19 (Dynabeads) were obtained from Dynal (Oslo, Norway).

Lymphocyte marker analysis

Peripheral blood mononuclear cells (PBMC) were first allowed to ingest latex particles for 60 min at 37°C to label the monocytes. B cells were identified by staining for surface immunoglobulin with the $F(ab)_2$ fraction of FITC-labelled goat anti-human immunoglobulin (Ortho). T lymphocytes, T helper–inducer lymphocytes, T suppressor–cytotoxic lymphocytes and NK lymphocytes were identified by staining with OKT3, OKT4, OKT8 and Leu11b, respectively, followed by incubation with FITC-labelled sheep anti-mouse IgG (Silenus, Dandenong, Australia) or FITC-conjugated goat anti-mouse IgM (Caltag, San Francisco, CA). Percentages of stained cells were obtained by counting at least 200 cells by u.v. microscopy.

Lymphocyte stimulation assays

PBMC (5×10^4) in 200 μl tissue culture medium (RPMI 1640 supplemented with L-glutamine, antibiotics and pooled human serum) were incubated in flat-bottomed microtitre trays with phytohaemagglutinin P (PHA; Difco) at 5, 10 and 20 $\mu g\ ml^{-1}$; concanavalin A (ConA, Sigma) at 20, 40 and 80 $\mu g\ ml^{-1}$; pokeweed mitogen (PWM, Sigma) at 0·5, 1 and 2 $\mu g\ ml^{-1}$; OKT3 at 6, 12, 25 and 50 $ng\ ml^{-1}$; or with no added stimulant. Quadruplicate cultures were set up for each dose of mitogen. ^{3}H-thymidine (0·2 μCi, specific activity 2·0 Ci/mmol) was added to each well at the initiation of the cultures, which proceeded for 3 days (PHA, ConA, PWM) or 4 days (OKT3) at 37°C, 5% CO_2, 100% humidity. Cells were harvested onto filter mats and incorporated radioactivity was determined by scintillation counting. Values are expressed as ct/min per 10^6 cells cultured.

Plaque-forming cell (PFC) assays

One-million unfractionated PBMC were stimulated with PWM, 1 $\mu g\ ml^{-1}$, in 1 ml RPMI 1640 + 5% human AB serum in 5 ml plastic tubes at 37°C, 5% CO_2, 100% humidity for 7 days. PWM stimulated cultures were also set up containing various combinations of purified B cells, plastic adherent cells, $CD4^+$ cells and $CD8^+$ cells from the patient or a normal control. Briefly, the procedure for obtaining these subsets was as follows: PBMC were incubated in plastic dishes for 45 min at 37°C and non-adherent cells removed. Adherent cells were collected by gently scraping with a rubber policeman. Non-adherent cells were then mixed with anti-CD19-coated Dynabeads. The B cells that bound to the beads were collected by applying a magnet along the tube. This method of positive selection has been shown not to cause any inhibition of B cell function (Funderud *et al.*, 1990). Anti-CD8-coated Dynabeads were then added to the adherent cell-depleted, B cell-depleted PBMC to obtain $CD8^+$ cells; finally anti-CD4-coated Dynabeads were used to purify $CD4^+$ cells from PBMC depleted of adherent cells, CD19, and CD8. The positively selected subsets were incubated overnight at 37°C, 5% CO_2 in RPMI 1640 + 5% AB serum, during which time Dynabeads were modulated off the cell surface and B cells, $CD4^+$ cells and $CD8^+$ cells could be collected by again applying the magnet to the side of the tube to attract the free Dynabeads. Co-cultures contained previously determined optimal numbers of subsets for PFC induction: these were 10^5 B cells, 2×10^5 $CD4^+$ cells, $0{\cdot}5 \times 10^5$ adherent cells and 10^5 $CD8^+$ cells/ml. Adherent cells were > 90% monocytes as judged by restaining with OKM1; B cells were > 95% pure on restaining for surface immunoglobulin; $CD4^+$ cells were > 95% $OKT3^+$, < 5% $OKT8^+$; $CD8^+$ cells were > 95% $OKT3^+$, < 5% $OKT4^+$. Following culture with PWM, numbers of PFC/10^6 cells harvested were determined using the protein A haemolytic plaque assay as previously described (Jones, 1981).

NK cell function

K562 cells were labelled with ^{51}Cr and 10^4 cells dispensed into round-bottomed microtitre trays. PBMC were added to give effector : target ratios of 5 : 1, 10 : 1, 20 : 1, 40 : 1 and 80 : 1. Further wells contained no effector cells (spontaneous release) or target cells plus detergent (maximum release). The medium was RPMI 1640 + 10% fetal calf serum (FCS) and the total volume was 200 μl. Each condition was set up in quadruplicate. After a 4-h incubation at 37°C, 5% CO_2, plates were centrifuged and 100 μl supernatant were taken for gamma counting. Percentage of

cytotoxicity for each effector:target ratio was calculated as follows:

$$\frac{\text{Experimental} - \text{Spontaneous release}}{\text{Maximum} - \text{Spontaneous release}} \times 100$$

Neutrophil function assays

Phagocytosis and killing of *C. albicans* by blood neutrophils and neutrophil chemotaxis responses to zymosan-activated human serum and FMLP were measured by standard techniques (Lehrer & Cline, 1969; Nelson, Quie & Simons, 1975). Neutrophil reduction of NBT was measured in EDTA-anti-coagulated blood by the slide test (Levinsky *et al.*, 1983), using *E. coli* endotoxin and phorbol myristate acetate (PMA) as stimulants. Neutrophil chemiluminescence (CL) in response to soluble and particulate stimulants was performed by the whole-blood method (Descamps-Latscha *et al.*, 1982) using heparinized blood diluted 1/20 in HBSS. CL was amplified by the presence of 10^{-3} M 5-amino-2, 3 dihydro-1,4-phthalazinedione (Luminol, Sigma), and stimulants were PMA 100 ng/ml and opsonized zymosan 0·2 mg/ml. Cells were kept at 35°C and CL was measured in a single-channel luminometer (LKB model 1250). The peak CL (mV) was recorded. Based on the blood neutrophil count, the peak CL/10^6 neutrophils was determined. This value was then divided by the equivalent peak CL given by normal control blood and the final result expressed as the peak CL index.

Measurement of adhesion glycoprotein on leucocyte cell membranes

Expression of leukocyte adhesion glycoprotein in neutrophil cell membranes was initially assessed by indirect immunofluorescence (IIF). The first antibody was OKM1 and the second was FITC-conjugated sheep anti-mouse IgG. For flow cytometry, neutrophils and mononuclear cells were separated from heparinized blood by bouyant density centrifugation on a discontinuous Ficoll–Hypaque gradient; lower layer SG 1.114, upper layer SG 1.077 (Kalmar *et al.*, 1988). The isolated neutrophil and mononuclear fractions were washed, adjusted to 10^7 cells/ml and labelled with appropriate MoAbs (anti-CD11a, CD11b or CD18) followed by incubation with FITC-conjugated sheep anti-mouse IgG. Labelled cells were examined by flow cytometry in a FACScan (Beckton Dickinson), equipped with an argon laser. Fluorescing neutrophils or lymphocytes were separated from contaminating cells and cell debris by electronic gating. Cells labelled only with FITC-conjugated second antibody were used to provide a background level of fluorescence for the method. Green fluorescent emission (510–550 mm) was recorded; channel number of the peak fluorescence shown on the histogram was converted to arbitrary arithmetic units and background fluorescence was subtracted to give net fluorescence, a measure of the cell membrane expression of the relevant antigen. A normal control was included in each assay as a reference, so that results from the patient and her family members could be expressed as a percentage of the normal control. In one experiment, isolated neutrophils were also exposed to FMLP, 10^{-6} M, for 30 min at 37°C to determine whether the leucocyte cell membrane expression of adhesion glycoproteins could be up-regulated.

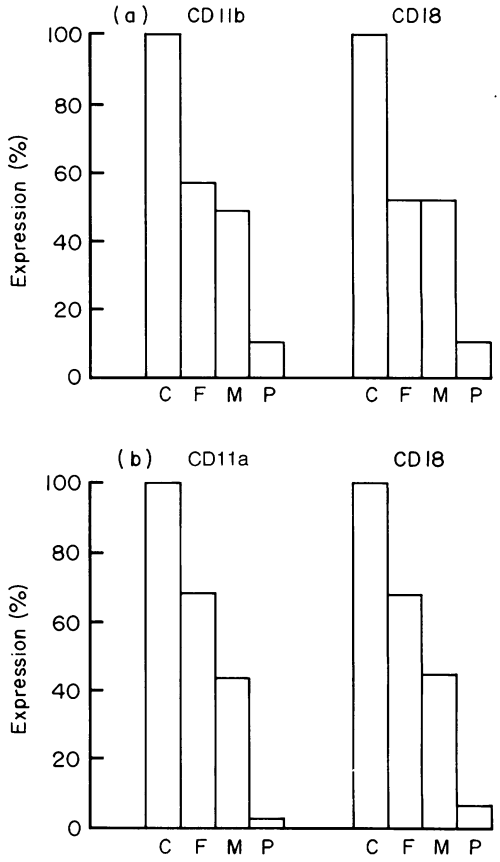

Fig. 1. Expression of leucocyte adhesion glycoprotein chains on neutrophils (a) and lymphocytes (b), quantified by flow cytometry and expressed as a percentage of the same marker on normal control cells (C, control; P, patient; F, patient's father; M, patient's mother).

RESULTS

Flow cytometry and immunofluorescence

Neutrophil screening tests clearly demonstrated a reduced expression of CD11b by IIF. Consequently, flow cytometric studies were carried out and blood neutrophils and lymphocytes from the patient were found on four occasions to express a low but significant reactivity with MoAbs specific for CD11b, CD18 and CD11a (Fig. 1). Both parents were found to express intermediate levels of the CD11b and CD18 on neutrophils and CD11a and CD18 on lymphocytes (Fig. 1). Incubating the peripheral blood neutrophils with 10^{-6} M FMLP for 30 min increased the expression of CD11b in the control by 160% and by 50% in the father, but there was no increase in the patient (Fig. 2). The lymphocyte subset profile is shown in Table 1. Absolute numbers of all subsets were raised.

Neutrophil function

Table 2 shows the results of the neutrophil function tests. Chemotaxis was reduced in response to both ZAS and FMLP. Phagocytosis of Candida and killing of Candida were both subnormal. The leucocyte CL response to a soluble stimulant (PMA) was greater than the control, but the response to a particulate stimulant (OZ) was only half that of the control's leucocytes.

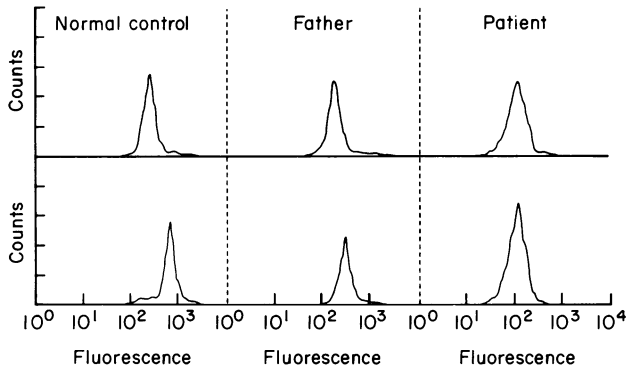

Fig. 2. Enhancement of neutrophil membrane CD11b expression by FMLP (top, unstimulated; bottom, FMLP 10^{-6}M).

Table 1. Lymphocyte subset profile of a patient with leucocyte adhesion deficiency

	Patient	Normal range
Leucocyte (μl^{-1})	83780	4000–10000
Lymphocytes (μl^{-1})	9216	800–3400
Surface immunoglobulin (%)	21	4–15
Surface immunoglobulin (μl^{-1})	1935	65–265
CD3 (%)	75	61–88
CD3 (μl^{-1})	6912	810–2214
CD4 (%)	54	33–61
CD4 (μl^{-1})	4977	500–1360
CD8 (%)	20	20–49
CD8 (μl^{-1})	1843	290–1030
CD4:CD8	2·70	0·9–2·7
CD16 (%)	15	8–24
CD16 (μl^{-1})	1382	195–451

Table 2. Neutrophil function tests

	Patient	Control	Normal range
Phagocytic index (Candida)	1·97	2·94	2·2–6·1
Killing of Candida (%)	13*	30	21–44
Chemotaxis (μM)			
ZAS	530	1550	600–1600
FMLP	370	1240	850–1270
NBT test (%)			
Unstimulated	61†	4	1–72
Endotoxin	96	89	52–86
Peak CL index			
PMA	1·65	1·0	—
Opsonized zymosan	0·52	1·0	—

* Killing assay performed in the presence of autologous plasma. Candida killing was equally impaired in the presence of control plasma.
† High unstimulated NBT score due to infection.

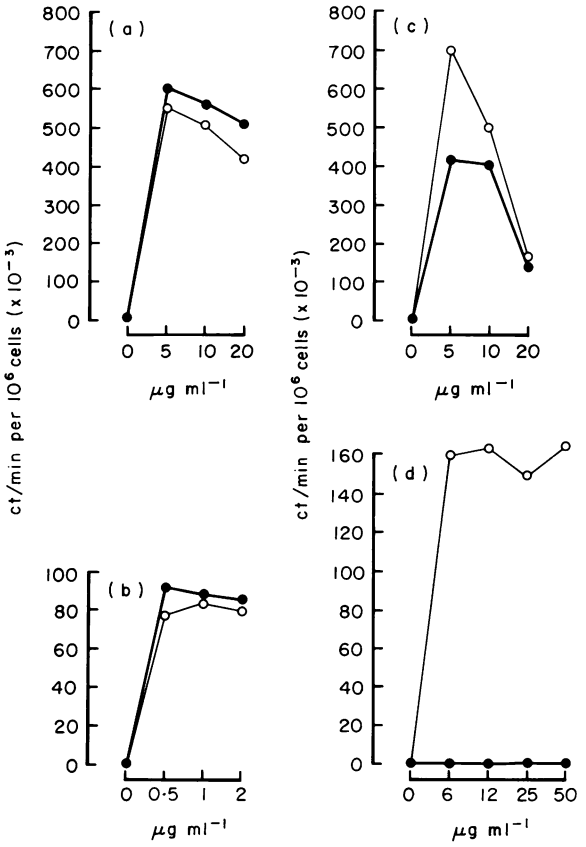

Fig. 3. Proliferative responses of PBMC from the LAD patient (●) and from a normal control (○) to PHA (a), PWM (b), ConA (c), and OKT3 (d).

Lymphocyte function

The proliferation of lymphocytes to various mitogens at different concentrations is shown in Fig. 3. Responses to PHA, ConA and PWM were normal, but the response to OKT3 was shown on two occasions (at 12 and 30 months of age) to be defective. Plasma immunoglobulin levels were normal for IgG and IgA, and raised for IgM. The patient had an adequate increase of viral titre after HSV1, parainfluenza type 2 and adenovirus infection. PWM stimulated PFC responses were assayed on two occasions and showed broadly similar results. The most recent results for the patient and her mother are shown in Table 3. Spontaneous NK cell activity was markedly diminished (Fig. 4).

DISCUSSION

Phenotypically, LAD can be divided into severe or moderate on the basis of clinical severity and of the quantity of the three heterodimers expressed. Patients with severe and moderate phenotypes expressed <0·3% and 2·5–31% of the normal amount of these molecules, respectively (Anderson *et al.*, 1985; Anderson, 1987). Our patient had about 12% of the normal level of CD11b and CD18 on her neutrophils. Chemotactic agents such as FMLP induce a rapid increase of the surface expression of CD11b on normal neutrophils (Anderson *et al.*, 1985), but our patient failed to respond in this way. The lack of upregulation of Mac-1 (CD11b) leads to inability of patient's neutrophils

Table 3. Plaque-forming cell (PFC) assay

PWM-stimulated culture	PFC/10^6 (% of control value) IgG	IgA	IgM
(C)B+(C)T_H+(C)APC*	9000 (100)	6000 (100)	4600 (100)
(P)B+(C)T_H+(C)APC	2000 (22)	1600 (27)	3200 (70)
(M)B+(C)T_H+(C)APC	3200 (36)	4800 (80)	2400 (52)
(C)B+(P)T_H+(C)APC	0 (0)	0 (0)	0 (0)
(C)B+(M)T_H+(C)APC	4200 (47)	2500 (42)	2300 (50)
(C)B+(C)T_H+(P)APC	3800 (42)	4000 (67)	2200 (48)
(C)B+(C)T_H+(M)APC	12200 (136)	8400 (140)	6800 (148)
(C)B+(C)T_H+(C)APC+(C)T_S	4400 (49)	3200 (53)	1800 (39)
(C)B+(C)T_H+(C)APC+(P)T_S	4400 (49)	2400 (40)	3000 (65)
(C)B+(C)T_H+(C)APC+(M)T_S	3800 (42)	2000 (33)	2000 (43)
Unfractionated (C) PBMC	14250 (100)	18000 (100)	8250 (100)
Unfractionated (P) PBMC	100 (0·7)	100 (0·6)	1800 (22)

C, control; P, patient; M, mother of patient; B, B cell; T_H, $CD4^+$ cell; T_S, $CD8^+$ cell; APC, antigen-presenting cell; PBMC, peripheral blood mononuclear cells.

* (C)B+(C)CD4, (C)T_H+(C)APC and (C)B+(C)APC gave 0 PFC/10^6.

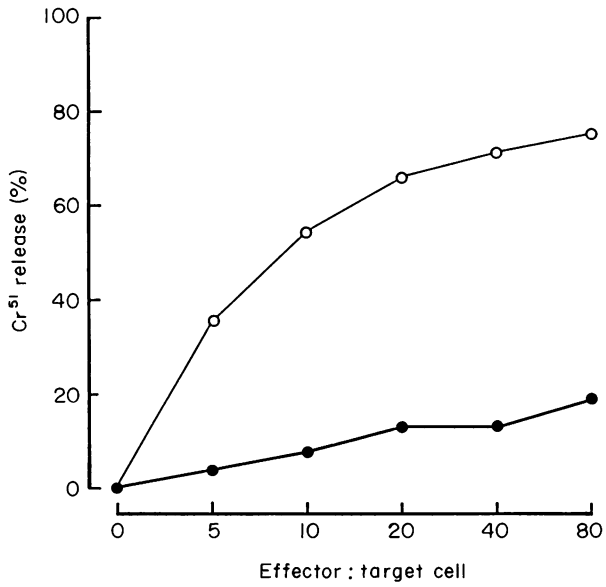

Fig. 4. NK cell function of PBMC from the LAD patient (●) and from a normal control (○).

to adhere to vascular endothelium, egress and migrate to sites of inflammation (Anderson, 1987). Expression of CD11a and CD18 on her lymphocytes were also low, at 3–7% of normal. Her parents were healthy heterozygotes with 44–68% of the normal level of LFA-1 and Mac-1. Her brother's neutrophils expressed normal level of CD11b. The results of the family study showed that LAD is of autosomal recessive inheritance, as reported (Anderson, 1987).

This patient presented with the characteristic features of LAD (Anderson *et al.*, 1985; Anderson, 1987), most of which can be explained by defects in adhesion-dependent neutrophil functions (Anderson *et al.*, 1984), which predispose the patient to bacterial and fungal infections. However, patients with LAD can handle viral infections, despite *in vitro* T cell, NK cell and monocyte defects (Kohl *et al.*, 1984; Fischer *et al.*, 1985, 1986; Krensky *et al.*, 1985). Neutrophils in our patient were moderately defective in adhesion-dependent functions, such as respiratory burst response to opsonized-coated particles and chemotaxis. Phagocytosis was marginally low and Candida killing moderately defective. Reduced killing presumably reflects reduced phagocytosis of the opsonized Candida. Reduced killing of Candida was observed in the presence of both autologous and control plasma, excluding an opsonic defect.

Despite her protracted diarrhoea, no stool pathogen was isolated but there was serological evidence that it might be due to adenovirus. Our patient had documented HSV1, RSV and parainfluenza virus type 2 infections from which she recovered uneventfully. This testified to her ability to handle viral infections and mount an adequate humoral response. Our patient's problem was secondary bacterial infection complicating a viral infection, such as the chronic cellulitis following her primary HSV1 gingivostomatitis. This was seen in a patient with the severe phenotype who died from a picornaviral tracheobronchitis complicated by extensive secondary bacterial and fungal infection (Anderson *et al.*, 1985). Therefore, patients with LAD are susceptible to complications of viral infections although they do not have a significant defect in mounting an immune response to viruses *in vivo*.

Although our patient had no problem in dealing with viral infections, she had marked defect in NK cell activity and lymphocyte proliferative response to OKT3. The patient's T lymphocytes proliferated normally in response to plant lectins, but failed to respond to OKT3. The difference between these two types of stimuli is that the former induces proliferation of both virgin and memory T cells while the latter stimulates memory T cells only (Byrne, Butler & Cooper, 1989). Our findings suggest a role for LFA-1 in the generation of memory T cells. However, the patient had a further increase in IgG titre against HSV following a recurrent HSV infection and strongly positive delayed-type skin hypersensitivity to Tetanus toxoid, suggesting intact *in vivo* memory T cell function. The reasons for the poor response to OKT3 remain uncertain, but could have been due to failure of cross-linking of CD3 antigens on T cells by anti-CD3 MoAb, which is normally mediated by monocytes (Umetsu *et al.*, 1987).

Although the patient's serum immunoglobulin levels were high normal and she had normal *in vivo* humoral response to various viruses, our patient had defective co-operation between $CD4^+$ cells, monocytes and B cells in PWM-induced *in vitro* generation of antibody secreting cells. Partial defects in accessory cell and B cell function were seen in the *in vitro* system, T suppressor function was normal. However, T helper function was completely deficient, with no induction of PFC by the patient's $CD4^+$ cells cultured with control B cells+adherent cells. Induction of IgM/PFC by PWM in unfractionated PBMC was less affected than IgG or IgA PFC, possibly because IgM production is less dependent on T cell help than IgA or IgG production. The mother's accessory cell and $CD8^+$ cell function appeared comparable to if not better than the control. However,

her CD4+ cell and B cell function was less than that of the normal control. There is an important role for LFA-1 in the cognate interactions between helper T cells and B cells (Noelle & Snow, 1990) but the defective *in vitro* interactions demonstrated in our case probably had little or no *in vivo* consequences, since she mounted good responses to various viruses *in vivo*. In fact, in our case the response to PWM did not reflect adequately the *in vivo* humoral response capability.

The T cell adhesive pathway involving CD2 and LFA-3 molecules (Makgoba, Sanders & Shaw, 1989) may compensate for a partial LFA-1 deficiency *in vivo* but not *in vitro*. Nevertheless, there may be some *in vivo* consequences of the lack of LFA-1 expression. It was shown that the transfer into neonatal mice of human LFA-1 negative lymphocytes did not protect them against herpes virus, whereas LFA-1 positive lymphocytes did (Kohl *et al.*, 1986). More importantly, it has been observed that patients with the severe phenotype of LAD did not reject HLA partially incompatible bone marrow transplants (Fischer *et al.*, 1988), probably secondary to defective interaction of host cytotoxic cells with donor marrow cells. Patients with moderate phenotype of LAD can reject haploidentical bone marrow transplants.

Kishimoto *et al.* (1987a) have classified the mutations of the β subunit gene into five different types. It has been shown that interferon-gamma (IFN-γ) and tumour necrosis factor-α (TNF-α) could enhance the transcription rate of β subunit gene but not the cell surface expression of the heterodimer in a patient with the moderate phenotype belonging to class V of Kishimoto's classification (Dimanche-Boitrel *et al.*, 1989). The problem was thought to lie in the poor association between α and β chains, leading to decreased expression at the membrane. It is essential to see whether these cytokines can help class II patients, who have defective RNA transcription or processing (Kishimoto *et al.*, 1987a), by increasing not only the β mRNA level but also the membrane expression of the heterodimer. Cytokines may then have therapeutic implication as in patients with chronic granulomatous disease (Ezekowitz *et al.*, 1988; Sechler *et al.*, 1988). Classification of patients according to mutation types will then become necessary to direct therapeutic choice. The recommended therapy is supportive for the moderate phenotype, with bone marrow transplant reserved for the severe phenotype (Fischer *et al.*, 1988), but there may come a time when the risk of bone marrow transplant is low enough to recommend this modality even for patients with the moderate phenotype who may die between 12 and 32 years of age (Fischer *et al.*, 1988). Another therapeutic option in the future will be somatic gene therapy, since Wilson *et al.* (1990) successfully corrected the functions of CD18-deficient lymphocytes by retrovirus-mediated gene transfer.

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