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Defective membrane expression of the LFA-1 complex may be secondary to the absence of the β chain in a child with recurrent bacterial infection*

Membrane and intracellular processing of the LFA-1 macromolecular complex, known to be involved in cytolytic function of T lymphocytes, was investigated in a child with recurrent bacterial infections, impaired natural killer activity, T cell-mediated lymphocytolysis and absent adhesion and migration of phagocytic cells. Monoclonal antibodies to the LFA-1 α and β subunits, able to precipitate the LFA-1α, 180-kDa chain, the p151 chain and β 94-kDa chain (shared by both α chains), were used in immunoprecipitation studies of patient and control phytohemagglutinin-blasts. Neither of the α chains nor the β chain were found in precipitates obtained from ^{125}I-surface-labeled patient cells in contrast to controls. However, the precursor of the LFA-1 α chain, a 170-kDa polypeptide, was identified in lysates of biosynthetically labeled patients' cells. These results suggest that the defective membrane expression of the LFA-1 complex may be secondary to the absence of the mature β chain.

1 Introduction

A family of three human leukocyte differentiation antigens, LFA-1, Mac-1 and p150, has been recently characterized biochemically and related to certain leucocyte functions [1–4]. The three molecules have a very similar subunit structure. They contain different α chains (the LFA-1 αL = 180 kDa, Mac-1 αM = 165 kDa and p150 αX = 150 kDa) and an identical β chain (94 kDa). The subunits are noncovalently associated in ($\alpha_1\beta_1$) structures expressed on the cell membrane. On normal T blasts, the LFA-1 antigen is detected, but not the Mac-1 [4]. Monocytes and granulocytes express all three antigens in different amounts.

Monoclonal antibodies (mAb) to human LFA-1 block antigen-specific cytotoxic T lymphocyte-mediated killing, and natural killer (NK) function [1]. The mAb to human Mac-1 blocks adhesion by myeloid cells, mediated by the complement receptor type 3 (CR3) to C3bi-coated particles [2]. Mac-1 may thus be identical to the CR3.

We studied a patient, born from fourth degree-related parents, with delayed umbilical cord detachment, severe recurrent bacterial infections, inability to form pus and marked leukocytosis [5]*. We found that (a) her neutrophils were defective in adherence, random migration chemotaxis and oxidative burst; (b) NK activity and cell-mediated lymphocytolysis were virtually absent; and (c) the LFA-1 membrane glycoproteins were not detected on T blasts, polymorphonuclear cells (PMN) or monocytes by immunofluorescence using specific mAb to the α and β chains. PMN and monocytes' C3bi receptors, tested by rosette formation with sheep erythrocytes coated with C3bi, were absent*. Similar or less profound deficiencies have been described by others [6–11].

To investigate the mechanism(s) underlying the absence of membrane expression of the LFA-1 macromolecular complex, we studied the biosynthesis of the LFA-1 subunits in patients' lymphoblasts. We have chosen to study phytohemagglutinin (PHA)-induced blasts since in man the LFA-1 complex is well expressed on activated T cells.

2 Materials and methods

2.1 Cell preparation, culture and labeling

Patient and control peripheral blood leukocytes prepared by Ficoll-Hypaque gradient centrifugation were cultured in presence of PHA (Gibco Inc., Grand Island, NY, 1/750 dil.) in RPMI 1640 + 10% human AB serum. At day 4, the nonadhering cells were collected, washed and incubated either in RPMI medium without serum (for the surface-labeling) or in the methionine-free minimum essential medium (MEM; Difco Laboratories, Detroit, MI), for 90 min at 37 °C. Percentages of blasts were comparable in the patient and control cultures (59 and 61%, respectively). Control cells (10^7) and 1.5×10^7 of patient cells were then surface-labeled with ^{125}I (Iodogen, Pierce Chemical Co., Rockford, IL) [12]. Another 5 × 106 of control or patient blasts were pulsed with 200 μCi/ml = 7.4 MBq/ml of [^{35}S]methionine (Amersham, GB; 500 Ci/mmol) in methionine-free MEM + 10% dialyzed fetal calf serum and chased in complete medium either for 16 h, or for the times indicated in the legend to Fig. 3. Surface- or metabolically labeled cells were lysed in 0.5% Nonidet-P40 in 10 mM Tris-HCl buffer, pH 8.0 + 2 mM phenylmethylsulfonyl fluoride and centrifuged at 11 000 × g for 10 min. Supernatants were then centrifuged at 105 000 × g for 60 min with or without prior addition of 1% sodium deoxycholate. Incorporated radioactivity was measured and supernatants were kept at –70 °C until use.

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Abbreviations: NK: Natural killer **PHA:** Phytohemagglutinin **mAb:** Monoclonal antibody(ies) **NMS:** Normal mouse serum **2D gel:** Two-dimensional gel analysis

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2.2 mAb and immunoprecipitation

The mAb TS 1/22 to the 180-kDa α chain and TS 1/18 to the 94-kDa β subunit of LFA-1 have been described [4]. The mAb 25.3.1 is directed to the α chain of LFA-1. Affinity-purified rabbit anti-mouse Ig (RaMIg) antibodies were coupled to Sepharose 4B (Pharmacia, Uppsala, Sweden).

Patient and control lysates obtained from cells with or without chasing were adjusted to contain equal (between patient and control) amounts of radioactivity and subjected to immunoprecipitation. Preclearing of lysates was obtained by the addition of 2 μl of normal mouse serum (NMS) followed by 50 μl of a 10% suspension of formalin-fixed *Staphylococcus aureus* or of 100 μl of 10% suspension of affinity-purified RaMIg coupled to Sepharose 4B. The supernatants were then incubated for 3 h at 4 °C either with 2 μl of NMS, 100 μl of culture supernatant of mAb 25.3.1 anti-α chain or with 20 μl of 1 : 100 TS 1/22 or TS1/18 ascites. To isolate immune complexes, 100 μl of RaMIg-Sepharose-4B were added and incubated for 3 h at 4 °C. Washed precipitates were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (12.5%) [13] under reducing conditions. In the two-dimensional gel analysis (2D gel), the first dimension was an isoelectrofocusing, and the second a 10% polyacrylamide slab gel electrophoresis [14]. Gels were fluorografed, dried and exposed to Kodak AR film for 5 to 10 days.

3 Results

3.1 Analysis of surface-labeled cells

A comparable proportion of blasts was found in patient and control cultures, and the amounts of ^{125}Iodine incorporated were $2.3 \pm 0.5 \times 10^6$ cpm, $3.0 \pm 0.3 \times 10^6$ cpm and $2.9 \pm 0.4 \times 10^6$ cpm per 10^6 cells from the patient, her father and control, respectively (average of 3 experiments).

Neither of the three polypeptides, 180 kDa, 150 kDa nor 94 kDa subunits, were found in the immunoprecipitates obtained either with anti-LFA-1 α chain (mAb 25.3 or TS 1/22) or with mAb TS 1/18 anti-β chain from the ^{125}I-surface-labeled cell lysate of patient (Fig. 1A, n and i) contrasting with normal

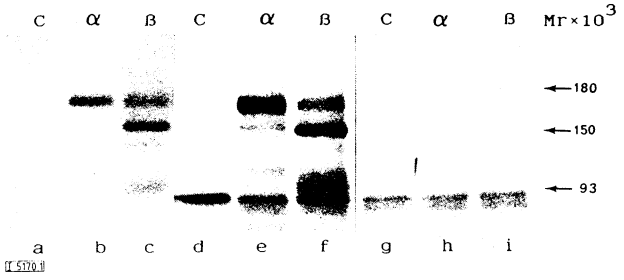

Figure 1. Patient, father and control LFA-1 subunits precipitated from lysates of surface-iodinated PHA-blasts. (a), (d) and (g) are control NMS precipitates; (b), (e) and (h) precipitates with TS 1/22 anti-α chain; (c), (f) and (i) precipitates with TS 1/18 anti-β chain obtained from controls (a–c); patient's father (d–f) and patient (g–i) blasts. Molecular mass standards used in this and other experiments were myosin, β-galactosidase and phosphorylase. Arrows indicate calculated molecular masses.

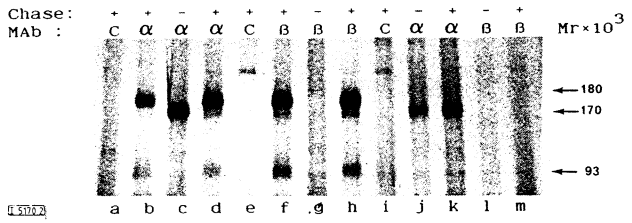

Figure 2. Patient and control LFA-1 subunits precipitated from the lysates of [^{35}S]methionine pulsed only or pulse-chased PHA blasts (5×10^6 cell equivalent per precipitate). Chase of 16 h is indicated at the top of the figure. (a), (e) and (i) are nonspecific NMS precipitates; (b–d) and (j, k) precipitates with TS 1/22 anti-α chain; (f–h) and (e, m) precipitates with TS 1/18 anti-β chain obtained from lysates of controls (a–h) or patient (i–m) blasts.

detection of class II antigen in the same lysate (results not shown). All three chains were detected in the patient's father's cell lysate (Fig. 1A, e and f) as they were in the control cell lysate (Fig. 1A, b and c).

3.2 Analysis of the biosynthetically labeled cells

The amounts of [^{35}S]methionine incorporated by 10^6 labeled but not chased lymphoblasts were 1.2×10^6 cpm and 2.48×10^6 cpm for the patient and the control, respectively. The amounts of [^{35}S]methionine after the chase of 16 h were 0.96×10^6 cpm and 1.7×10^6 cpm for the patient and the control, respectively. Similar differences in the radioactivity incorporated by patient and control blasts was observed for all chase times.

Equal amounts of radioactivity were used in each precipitation, and the mAb were added in excess in amounts previously found to be sufficient to precipitate all the LFA-1 subunits from a lysate of 2×10^7 PHA-induced, iodinated control blasts.

Under these conditions, when the [^{35}S]methionine-labeled blasts of the patient and control were lysed before or after 16 h of chase and then the lysates were precipitated by specific antibodies, the results were as follows. The mAb TS1/22 readily detected in the lysate of control cells the α chain of 170 kDa before chase and of 180 kDa after chase (Fig. 2c compared to b and d). The same antibody precipitated only the precursor form of the α chain, a 170-kDa polypeptide from lysates of patient's chased or not chased blasts (Fig. 2j and k). The TS 1/18 anti-β chain mAb detected no specific LFA-1 peptides in the unchased blasts of control (Fig. 2g) but readily detected both the 94-kDa β chain and 180-kDa α chain in the lysates from chased blasts of two controls (Fig. 2f and h). TS 1/18 antibody detected neither β nor α subunits in the lysates from patient's blasts whether or not they were chased (Fig. 2 l and m). A constant 170-kDa mobility throughout the chase of the patient's chain contrasting with sequential molecular mass increase of the control α chain was confirmed in a pulse-chase experiment (Fig. 3).

The anti-LFA-1 precipitates obtained from the lysates of control and patient unchased blasts were analyzed by 2D gel elec-

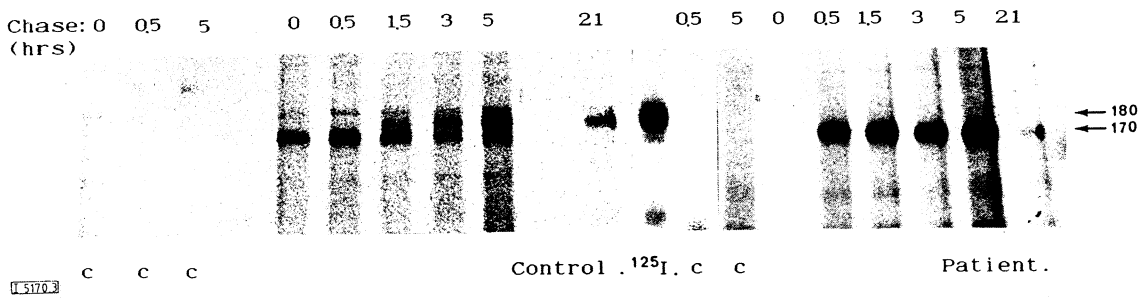

Figure 3. Patient and control LFA-1 α chains precipitated with TS 1/22 from [^{35}S]methionine pulse-chased PHA blasts. Two × 10^6 patient and 1 × 10^6 control cells/tube were pulsed for 25 min and chased for times indicated at the top of the figure. Precipitates from patient blast, right-side, from control, left-side; (c) are control NMS precipitates; ^{125}I, is precipitate obtained with mAb 1-22 from surface-iodinated control PHA blasts showing the position of surface α chain of the LFA-1. Part of precipitate from unchased cells from patient (0) was lost and no α chain is detected at this exposure time.

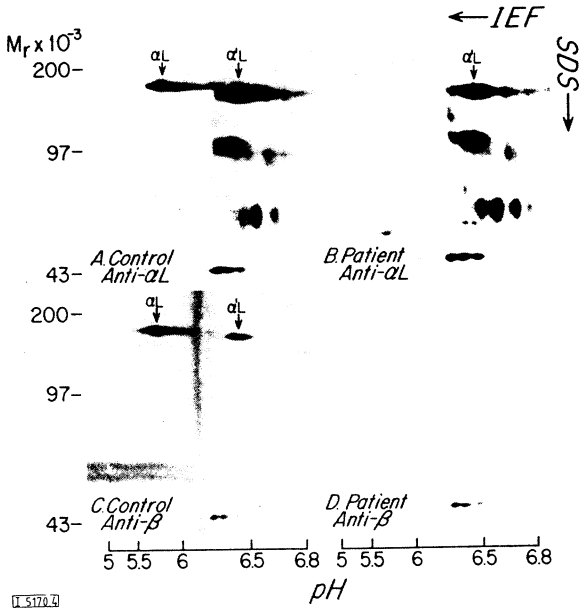

Figure 4. 2D gel analysis of patient and control LFA-1 subunits. Five × 10^6 patient and control PHA blasts were labeled with [^{35}S]methionine for 2 h and lysed without chase. The TS 1/22 anti-α (panels A and B) and TS 1/18 anti-β precipitates (panels C and D) were separated in the first dimension by isoelectric focusing and in the second in a 10% polyacrylamide slab gel.

trophoresis. The most acidic spot corresponding in the control anti-α TS 1/22 precipitate to a mature αL peptide (Fig. 4, panel A) was clearly lacking from similar precipitate of patient cell lysate (Fig. 4, panel B). All LFA-1 peptides were absent from the patient anti-β TS 1/18 precipitate (Fig. 4, panel D), while in the control precipitate TS 1/18 TS 1/18 mAb detected mature and precursor αL chains in the absence of visible β chain peptides (Fig. 4, panel C), as expected from previous experiments.

4 Discussion

Several patients with recurrent bacterial infections associated with deficiencies of granulocyte membrane protein have been described [6–11]. In the patient reported in this study, several leukocyte-mediated functions were abnormal, as described in detail elsewhere*.

To identify the molecular abnormalities of patients' lymphocytes we used the well-characterized mAb to the LFA-1 subunits. The mAb TS 1/22 is able to precipitate the α chain (180 kDa) and the β chain (94 kDa), when added to lysate, not treated with acid pH to dissociate the LFA-1 subunits [4]. Under similar conditions, mAb TS 1/18 was described to identify the LFA-1 αL and β chains in all T cell lines and, in addition, precipitate the p151 αX chain of 151 kDa in some but not all lines studied [1].

These antibodies detected all three subunits: the 180-, 151- and 94-kDa chains in the lysates of surface-iodinated PHA blasts of the control and the patient's father.

The same antibodies were unable to detect any of three LFA-1 polypeptides in the lysate of surface-labeled blasts of the patient, in spite of normal response of the patients' lymphocytes to PHA and normal detection of other surface antigens (class II, results not shown). However, the TS 1/22 anti-α mAb did detect clearly the LFA-1 170-kDa polypeptide in biosynthetically labeled patient cells. Whereas the molecular weight of the chain precursor of 170 kDa, detected by the TS 1/22 in the control cells, increased after the chase to an apparent molecular mass of 180 kDa, the patient's chain conserved a 170-kDa mobility before and after the chase. The 2D gel analysis showed that, indeed, an acidic α chain peptide was absent from the patient's lysate (Fig. 4). The 1/22 anti-α chain mAb is thus able to precipitate the α chain at all stages of its biosynthesis. In contrast, the TS 1/18 anti-β chain can apparently detect preferentially the mature β chains, since in control methionine-labeled, unchased blasts the β chain is hardly detected. This does not reflect merely a low methionine content, since the control 94-kDa β chain was well detected after the chase. Yet, the β chain may contain fewer methionine residues than the α chain: 1 × 10^6 cell equivalents were sufficient to detect the control αL chain but not the control chain despite the chase (Fig. 3A). Five × 10^6 cell equivalents were needed to detect the [^{35}S]methionine-labeled β chain. Although sufficient numbers of labeled patients' cells were used in several experiments as in the one shown in Fig. 2, the

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analysis of patients' LFA-1 subunits remains incomplete since a chain precursor, if synthesized, may not be detected using TS 1/18 mAb.

In spite of this limitation, it is evident that the LFA-1 subunit synthesis, assembly and membrane insertion is defective in this syndrome. Springer et al. studied the biosynthesis of LFA-1 subunits in 3 of 8 patients with a similar clinical presentation and biological findings [15]. They reported, as we do, a presence of the LFA-1 α chain precursor in patients' metabolically labeled blasts and an absence of the β chain in the same lysate. They concluded that impaired biosynthesis of the β molecule represents a fundamental molecular lesion in this syndrome. Neither Springer et al. nor we were in a position to isolate a precursor β chain from control unchased PHA blasts. Patients' β chain is either not synthesized at all or if so, it lacks the epitope towards which the TS 1/18 mAb is directed, as well as the one which allows the association with the α chain. The LFA-1 α and β subunits have been shown in U937 cell line to be separately synthesized, associated into an α-β complex and then proceed to acquire mature form [4]. The mode of synthesis and assembly of this macromolecular complex is similar to the other multigenic surface antigens such as immunoglobulins [16] and histocompatibility antigens [17]. For the major histocompatibility complex products, it has already been shown that the synthesized HLA class I heavy chain cannot be expressed on the membrane since the β_2-microglobulin mRNA is not transcribed and not because the heavy chains are not glycosylated [18]. Our patient's lymphocytes may represent an analogous situation. Although defective glycosylation of the patient's α chain is strongly suggested by our results, this may be a consequence of the absence of β chain. Indeed, the kinetics of LFA-1 and Mac-1 biosynthesis suggests that the association between the α and β chains triggers the processing of precursors [4, 19]. Further work is needed to determine whether any processing events are used to regulate physiologically the expression of LFA-1 complex in activated human T blasts. A fine difference of mobility between the surface-iodinated control αL chain and the metabolically labeled αL chain after 21 h of chase suggests a very late addition of some polar residues.

Taken together, the absence of β chain in the anti-α and anti-β precipitates, obtained from cytoplasm of patients' blasts, demonstrate that synthesized but not mature α chain is not associated to β chain and explains the absence of the α-β complex on the cell membrane. To decide whether a prime defect in this syndrom is a nontranscription of the β chain mRNA or rather a structural defect of one or both LFA-1 chains remains to be established using an antibody able to precipitate the β chain precursor.

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