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CURRENT TOPIC

Leucocyte adhesion deficiency

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Rare experiments of nature often lead to a better understanding of fundamental principles of human biology. The clinical description of a severe congenital immunodeficiency presenting with persistence of a 'fleshy' often infected umbilical cord ('delayed cord separation') and overwhelming septicaemias[1] has led to a rapid progress in our appreciation of the importance of leucocyte-leucocyte and leucocyte-endothelial interaction in health and disease.

Leucocyte adhesion deficiency (LAD), as the disease is now called, is a rare immunodeficiency characterised by severe recurrent and often fatal bacterial infections with a failure of pus formation and delayed wound healing.[2] A deficiency in leucocyte β_2 integrins was recognised to be the cause of this syndrome in the early 1980s.[3]

Background

The cellular elements of human immune system (polymorphonuclear leucocytes, monocytes/macrophages, T and B cells, and other types of leucocytes) are constantly communicating with each another. This network includes endothelial surfaces as well as extracellular matrices and leads to modulation of immune and inflammatory responses and to control of cell traffic (homing). Some of these interactions are dependent on cytokines for their regulation,[4] others require firm leucocyte-cell or leucocyte-matrix contact, called adhesion.[5] Adhesion events are often transient, follow cell activation and are an early step, for example in transendothelial migration of leucocytes, which is of a great biological relevance in host defence and tissue injury.[6]

Table 1 Families of adhesion molecules: the integrins

Receptor	*Tissue distribution*	*Ligand*
β_1 integrins		
VLA-1 (CD49a/CD29)	Activated lymphocytes, fibroblasts	Laminin, collagen
VLA-2 (CD49b/CD29, LPAM-2)	Activated T cells, platelets, fibroblasts, endothelium, epithelium	Laminin, collagen
VLA-3 (CD49c/CD29)	Fibroblasts, epithelium	Fibronectin, laminin, collagen
VLA-4 (CD49d/CD29, LPAM-1)	Lymphocytes, monocyte, fibroblast, neural crest derived cells	Fibronectin, VCAM-1
VLA-5 (CD49e/CD29)	T cells, thymocytes, platelets, fibroblasts, endothelium, epithelium	Fibronectin
VLA-6 (CD49f/CD29)	Fibroblasts	Laminin
β_2 integrins		
CD11a/CD18 (LFA-1)	Leucocytes	ICAM-1, ICAM-2
CD11b/CD18 (CR3, Mac-1, MO-1)	Granulocytes, monocytes, macrophages, large lymphocytes, CD5 +ve B cells	ICAM-1, C3bi, fibrinogen
CD11c/CD18 (p150,95; CR4)	Granulocytes, monocytes, macrophages, activated lymphocytes	C3bi
β_3 integrins		
CD41/CD61 (GPIIb-IIIa)	Platelets, megakaryocytes	Fibrinogen, vWf, fibronectin, thrombospondin
CD51/CD61 ('3)	Platelets, megakaryocytes	Vitronectin, vWf, thrombospondin, fibrinogen
β_4 integrins	Epithelial cells	CD21, TAPA-1, Leu-13
β_5 integrins	Epithelial cells	Fibronectin, vWF
β_6 integrins (LPAM-1)	Lymphocytes	Unknown
β_7 integrins	T lymphocytes	CD28

Classification of adhesion molecules

Three families of adhesion receptors (tables 1 and 2) are so far known,[7] and we will focus here on those related to LAD.

THE LEUCOCYTE INTEGRINS

The essential function of the integrins is the integration of the cytoskeleton with the extracellular environment and they are found on most cell types (tables 1 and 2). The β_2 integrin (CD11/CD18), a glycoprotein complex, is required for adhesion dependent functions such as aggregation, spreading on artificial substrates, chemotaxis, phagocytosis, cell mediated killing, and adherence to endothelium.[8] The various functions of this molecule have in common the need of the cell to adhere to a target in a reversible manner and are temperature dependent and coupled to the presence of divalent cations.[9] Each integrin consists of a distinct heavy (150–180 kDa) α polypeptide chain non-covalently linked to a light (95 kDa) β polypeptide chain (CD18) common to all three subunits: CD11a (lymphocyte function-associated antigen-1 (LFA-1) present on the surface of virtually all circulating leucocytes), CD11b (CR3, Mac-1, Mol, is limited to neutrophils, monocytes and natural killer cells) and CD11c (CR4, gp 150,95 has a similar distribution to CD11b).

Neutrophils from patients with the deficiency demonstrate in vitro defects in adhesion dependent functions that can be reproduced in normal neutrophils by addition of monoclonal antibodies directed to function related epitopes of the glycoprotein complex.[10] Monoclonal antibodies to CD11b and/or CD18 have been shown in vitro to prevent neutrophil aggregation and adherence to endothelial monolayers.[11] Unstimulated neutrophils express CD11b on the cell surface and even in greater quantities within the secondary and/or tertiary granules.[12] Upon stimulation, the contents of

Table 2 Families of adhesion molecules: the immunoglobulin and selectin superfamilies

Receptor	*Tissue distribution*	*Ligand*
Immunoglobulin superfamily:		
ICAM-1 (CD54)	Activated lymphocytes, monocytes, histiocytes, fibroblasts, endothelium, epithelium	CD11/CD18 (LFA-1, Mac-1)
ICAM-2	Endothelium, lymphocytes, monocytes	CD11a/CD18 (LFA-1)
CD2 (LFA-2)	Lymphocytes	CD58
CD58 (LFA-3)	Macrophages, T and B lymphocytes, endothelium	CD2
VCAM-1	Endothelium	VLA-4
CD3/TcR	T lymphocytes, γ/δ T cells	MHCI, II
CD4	T cell differentiation (thymus)	MHCII, gp120
CD8	T cells	MHCI
MHC class II	T lymphocyte	CD4
MHC class I	Natural killer cells, viral protein	CD8
CD28 (Tp44)	T cell, plasma cells	B7
CD21 (CR2)	B lymphocytes	C3d/EBV
CD35 (CR1)	Erythrocyte, neutrophil, monocytes, lymphocytes, macrophates	C3b, C4b
Selectin superfamily:		
L-selectin (mel-14, LAM-1, LECAM-1)	Leucocytes	E-, P-selectins
E-selectin (ELAM1)	Endothelium	SSEA-1, L-selectin
P-selectin (CD62, PADGEM, GMP-140	Platelets, endothelium	sLewis-X, L-selectin

Previously used or alternative names in brackets.

Table 3 Clinical features of LAD

Often persistent leucocytosis (12–160×10⁹/l)
Skin/subcutaneous infections
Indolent subcutaneous abscess or cellulitis
Cellulitis or abscess associated with wound or trauma
Gingivitis/periodontitis
Otitis media
Ulcerative stomatitis/pharyngitis
Systemic infection
Peritonitis
Perianal abscess
Pneumonia
Aseptic meningitis
Necrotising pharyngitis/tracheitis
Delayed umbilical cord severance/infection
Impaired wound healing and/or infection of surgical wounds

these granules are translated to the cell surface, resulting in a threefold to 10-fold increase in surface associated CD11b.[13] It has been suggested that this increase in surface expression possibly after conformational changes may play a part in the mechanism of enhanced neutrophil adhesiveness.[14]

Clinical manifestations and diagnosis

LAD syndrome is characterised by delayed umbilical cord separation, recurrent severe bacterial infection, periodontitis, delayed wound healing, and often persistent leucocytosis with absent pus formation. The clinical manifestation are summarised in table 3.

Patients with LAD present with recurrent and often life threatening infections. *Staphylococcus aureus*, group α/β haemolytic streptococci, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Escherichia coli* are common pathogens.[15] Recurrent otitis media, severe gingivitis, pharyngitis, stomatitis,[16] and perirectal abscesses are also common. Deep seated infections of the lungs, gastrointestinal tract, or nervous system may progress to overwhelming sepsis despite aggressive treatment.[17] Increased susceptibility to infections is directly related to inability of deficient phagocytes (both granulocytes and monocytes) to migrate to sites of tissue infection and to clear the offending organisms (through phagocytosis and cell mediated cytotoxicity). Delayed separation of umbilical cord in neonates and formation of dysplastic scars[18] due to secondary wound healing processes in older children are common manifestations. Intradermal skin testing is unaffected as is delayed hypersensitivity (Mantoux test). Increased susceptibility to viral infections, although theoretically possible, is not part of the clinical spectrum.

The clinical severity of the disease is often proportional to the degree of the deficiency. Patients with the complete deficiency state (type I) often die at an early age with overwhelming sepsis whereas those with the partial form (type II) of the disease may have a milder course and can survive into adulthood provided infections are treated early or reduced by continuous antibiotic prophylaxis.[19] Heterozygous individuals (parents) are asymptomatic. The recognition of a new case of LAD depends on the awareness of the physician of the possibility of a LAD in a patient with recurrent and serious infection in the presence of often *persistently* high leucocyte counts. The exact diagnosis is confirmed by examining the neutrophils for surface expression of CD11/CD18 molecules by immunofluorescence techniques and by flow cytometry.

Pathogenesis of LAD

Clinical manifestations of the LAD syndrome are related to the deficient function of CD11/CD18 molecules. The molecular defect is heterogeneous and ranges from defective gene expression with undetectable mRNA,[20] to aberrant gene splicing with production of an abnormal β chain, to point mutations in the highly conserved region of the β subunit that results in a precursor that is unable to associate with the α units.[21]

CD11/CD18 molecules are essential for phagocytic cell function (macrophages and monocytes) but the relative contribution of this heterodimer varies with the cell type. In neutrophils, binding with iC3b coated particles, spreading, aggregation, chemotaxis, neutrophil dependent adherence to the endothelium, and phagocytosis are largely mediated by CD11/CD18.[22] In monocytes, CD11/CD18 also facilitates iC3b binding and phagocytosis but not spontaneous or stimulus induced monocyte aggregation which is CD11a/CD18 dependent.[23] The monocyte and neutrophil CD11c/CD18 may contribute to monocyte binding to iC3b. Antibody dependent cytotoxicity and natural killer activities as well as endothelium dependent adhesion of phagocytes is mediated by all heterodimers.

In B and T lymphocytes, all CD11/CD18 dependent functions are mediated by CD11a/CD18.[24] Anti-CD11a/CD18 monoclonal antibodies inhibit homotypic and heterotypic interactions of B and T lymphocytes to endothelium and to other targets.[25] T cell proliferation in response to stimuli which require cell-cell contact is also inhibited.[26] These data account for impaired cell mediated cytotoxicity, lymphoproliferative responses, and antibody production in response to T cell dependent antigens seen in vitro.

The gene encoding the β subunit has been mapped to chromosome 21 at 21q22, which is a breakpoint in chromosomal translocations associated with blast crisis in chronic myeloid leukaemia.[27] An increase of leucocyte adhesiveness due to over expression of CD11a/CD18 has been reported in patients with trisomy 21 (Down's syndrome); its clinical relevance remains to be established.[28] All three α subunits have been localised to p11-p13.1 on chromosome 16. Inversions in translocations involving this region have been reported in patients with acute myelomonocytic leukaemia.[27]

The causes for delayed and defective wound healing are not well understood. Acute inflammation and polymorphonuclear infiltration of wound is the initial step of healing. Inability of macrophages to emigrate to wound site render the inflammatory reaction incomplete and/or inadequate, which may explain the delay in healing and formation of hypertrophic scars. Absence of inflammation, however, cannot be the only explanation for impaired wound healing as scarless healing of wounds due to surgery in utero (fetus), where neutrophil functions are down regulated,[29] is reported. The role of amniotic growth factors in this process needs to be established.

NEONATAL NEUTROPHILS

Human neonates are at high risk of infection, which is thought to be due to immaturity of their non-adaptive immune system. Studies in neonates have demonstrated diminished or delayed emigration of the neutrophil into extravascular inflammatory sites. Significant differences of neutrophil (chemotactic) functions compared with adults have been reported and marked reduction in the level of CD11b/CD18 on the surface of the neonatal neutrophil may be the cause for these functional abnormalities.[30] These abnormalities are transient in a healthy newborn and disappear with maturation.

Laboratory findings

Complete (0–2%, type I) or partial (>10–20%, type II) absence of the leucocyte adhesion molecule CD11/CD18 on the cell surface is diagnostic and also allows prenatal diagnosis for severe forms of LAD by cordocentesis in families with history of the disease.[31] Another characteristic finding is the persistent neutrophilia that is invariably seen even in infection-free periods. Deficient phagocytes are unable to ingest serum opsonised *particles* due, in part, to their inability to bind to complement iC3b, the major serum opsonic C3 fragment. The respiratory burst and degranulation responses associated with the process of phagocytosis are secondarily impaired. Stimulated random and directed migration, homotypic and heterotypic cell interactions are also impaired resulting in a paucity of phagocytes (but not lymphocytes) at infected sites in vivo. Adhesion independent functions such as superoxide generation and degranulation in response to *soluble* stimuli are normal.

Lymphocytes in LAD

Abnormalities in functions of affected lymphocytes have been found in vitro. Defects in antigen-mitogen or alloantigen-induced lymphoproliferation, cell mediated killing by T and natural killer cells, homotypic and heterotypic adhesion of B and T cells interactions, and T and B cell adhesion to endothelium are reported. Many of these defects are most apparent at low rather than high concentrations of a stimulus. At higher concentrations of the stimulus or during secondary stimulation, many of these functions become normal especially in patients with the partial deficiency. The above mentioned effects on T cell functions have been clinically exploited by incorporating the infusion of CD11a (LFA-1) antibodies into bone marrow transplantation protocols before and after transplantation. This policy has increased engraftment of haploidentical bone marrow grafts in patients with severe combined immunodeficiencies.[32] Prevention of T cell allorecognition via infusion of anti-CD11a monoclonal antibodies can also be used alone or in combination with other antibodies (for example CD3, CD4, CD8, CD25) in the treatment of graft versus host disease.

Conservative treatment

Early identification of the affected individuals of high risk families is most important for formulation of rational treatment strategies.

ANTIBIOTICS

The mainstay of conservative treatment is antibiotics given during infections and as a prophylaxis. A broad spectrum antibiotic (intravenous) should be commenced as soon as the necessary cultures (including fungal cultures) are taken. Adjustment of the antibiotic regimen can be made at a later time. In severe systemic infections not responding to antibiotic treatment, antifungal treatments need to be considered early as patients with LAD are *functionally* neutropenic. To avoid infections, strict dental hygiene is important and all surgical procedures should be performed under cover of appropriate broad spectrum antibiotics.

Patients with LAD should receive all vaccinations according to the current recommendations and injections should be given under strict antiseptic precautions.

Leucocyte transfusion and bone marrow transplantation

Granulocyte transfusion may be useful in selected cases of (localised) infections that cannot be controlled otherwise. Their use is limited because of difficulties in supply (daily donors) and of potential development of alloantibodies. At present, the only corrective

treatment that can be offered is a bone marrow transplantation, which allows generation of healthy neutrophils and other leucocytes.[33] Patients with complete (type I) LAD constitute a *lower* transplantation risk because of a reduced rejection rate.

Gene therapy

A more selective and curative approach involves somatic gene therapy through introduction into haematopoietic stem cells of the normal CD18 subunit using viral vectors[34] as currently being used for somatic gene therapy for adenosine deaminase deficiency.[35] A transfection rate of over 10% may be sufficient to convert type I to a less severe type (II) or even to a heterozygote state.

Counselling

LAD is transmitted in an autosomal recessive manner and affects patients of different ethnic background. Heterozygous carriers are asymptomatic. The likelihood of having an affected child is one in four and increases in consanguineous marriages. Gene mutation in a carrier mother has been reported and the child had severe form of the disease. Pregnant mothers with a positive history of the disease should be offered a cordocentesis at 18 weeks of gestation.

Prognosis

Type I LAD is invariably fatal, often within the first few years of life. Patients with partial (type II) deficiency may have a milder course and can survive into adulthood. Overwhelming infections, however, can also reduce the life span of patients with partial deficiencies. These patients need meticulous health surveillance and early initiation of antibiotic treatment.

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Notes