we set forth to identify viral genome in the endothelium of the vasculitic lesions. Cell-mediated immune (CMI) dysfunction, including defective natural killer cell function has been documented in patients with XLP, and defective CMI may result in altered EBV tissue tropism as we suggest. We agree that many more patients, as well as controls, would need to be studied to definitively answer this issue. The functionality of EBV-specific CTL remains a subject for debate.[8,9] Normal EBV-specific CTL function, as has been detected in some patients with XLP, might indeed explain the targeted assault and damage of the infected vascular structures that we have demonstrated.

Rusung Tan, Jan Dutz, Loralyn Benoit, Derek de Sa, and Anne Junker

Correspondence: Rusung Tan, Departments of Pathology and Laboratory Medicine, University of British Columbia and BC's Children's Hospital, 4480 Oak St, Vancouver, BC, Canada, V6H 3V4

References

1. Seemayer TA, Gross TG, Egeler RM, et al. X-linked lymphoproliferative disease: twenty-five years after the discovery. Pediatr Res. 1995;38:471-478.
2. Brandau O, Schuster V, Weiss M, et al. Epstein-Barr virus-negative boys with non-Hodgkin lymphoma are mutated in the SH2D1A gene, as are patients with X-linked lymphoproliferative disease (XLP). Hum Mol Genet. 1999;8:2407-2413.
3. Gross T, Kelly C, Davis J, Pirruccelllo S, Sumegi J, Seemayer T. Manifestations of X-linked lymphoproliferative disease (XLP) without evidence of Epstein-Barr virus (EBV) infection [abstract]. Clin Immunol Immunophathol. 1995;75:281.
4. Sumegi J, Huang D, Lanyi A, et al. Correlation of mutations of the SH2D1A gene and Epstein-Barr virus infection with clinical phenotype and outcome in X-linked lymphoproliferative disease. Blood. 2000;96:3118-3125.
5. Arico M, Imashuku S, Clementi R, et al. Hemophagocytic lymphohistiocytosis due to germline mutations in SH2D1A, the X-linked lymphoproliferative disease gene. Blood. 2001;97:1131-1133.
6. Jones K, Rivera C, Sgadari C, et al. Infection of human endothelial cells with Epstein-Barr virus. J Exp Med. 1995;182:1213-1221.
7. Weck KE, Dal Canto AJ, Gould JD, et al. Murine gamma-herpesvirus 68 causes severe large-vessel arteritis in mice lacking interferon-gamma responsiveness: a new model for virus-induced vascular disease. Nat Med. 1997;3:1346-1353.
8. Harada S, Bechtold T, Seeley JK, Purtilo DT. Cell-mediated immunity to Epstein-Barr virus (EBV) and natural killer (NK)-cell activity in the X-linked lymphoproliferative syndrome. Int J Cancer. 1982;30:739-744.
9. Rousset F, Souillet G, Roncarolo MG, Lamelin JP. Studies of EBV-lymphoid cell interactions in two patients with the X-linked lymphoproliferative syndrome: normal EBV-specific HLA-restricted cytotoxicity. Clin Exp Immunol. 1986;63:280-289.

To the editor:

Langerhans cells and the cells of Langerhans cell histiocytosis do not express DC-SIGN

Geissmann and colleagues recently reported that cells of Langerhans cell histiocytosis (LCH) in osseous and/or chronic lesions have an immature dendritic cell phenotype.[1] In addition to CD1a, LCH cells in all 25 cases examined expressed Langerin, a recently described C-type lectin that appears to be restricted entirely to Langerhans cells.[2]

An increasing number of dendritic cell lectins, such as dendritic cell–specific ICAM-grabbing nonintegrin (DC-SIGN), dendritic cell immunoreceptor (DCIR), DEC-205, and CD23, are now recognized.[3] We consider it important to determine whether these lectins are expressed on similar, overlapping, or mutually exclusive dendritic cell subsets, as such information may suggest the biological roles of the individual lectins. Our recent work has focused on DC-SIGN (manuscript submitted and Soilleux et al[4]), which has 32% sequence identity at the amino acid level to Langerin across the carbohydrate recognition domain. DC-SIGN has the capacity to bind the HIV surface molecule glycoprotein 120 in an inhibitable manner by mannose,[5,6] as well as the lymphocytic surface-expressed molecule ICAM-3,[7] and the endothelial surface molecule ICAM-2.[8] Langerin also possesses the critical mannose binding motif seen in DC-SIGN. It has not yet been determined whether HIV may interact with Langerin in a manner analogous to its interaction with DC-SIGN.

We have recently demonstrated that DC-SIGN is expressed by cells with an immature dendritic cell phenotype (manuscript submitted) similar to that described by Geissmann et al[1] in LCH cells. In our study, the DC-SIGN$^+$ cells were CD14^{low} HLA-DR$^{+/low}$ CD68$^+$ 8100$^{+/-}$ and only rarely expressed dendritic cell activation markers such as CD83, CD86, and cmrf-44 (manuscript submitted). Moreover, the DC-SIGN$^+$ cells were consistently negative for CDla (manuscript submitted). Cells matured in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor-α (TNF-α), transforming growth factor β (TGF-β), stem cell factor (SCF), and flt-3 ligand from CD34$^+$lin$^-$ peripheral blood cell precursors, to give a Langerhans cell phenotype,[9] showed no evidence of DC-SIGN expression by reverse transcriptase–polymerase chain reaction (RT-PCR).[4] Dendritic cells matured from peripheral blood monocytes in the presence of GM-CSF and interleukin-4 (IL-4) and could be shown to express DC-SIGN both by RT-PCR and fluorescence-activated cell sorting analysis (manuscript submitted and Soilleux et al[4]).

In view of the findings of Geissmann et al,[1] we have extended our analysis of the expression of DC-SIGN in relation to CDla on dendritic cells by examining 4 specimens of normal skin and 8 specimens of LCH obtained from the Department of Histopathology, Addenbrooke's Hospital, Cambridge, United Kingdom. Two of the LCH cases were from the retro-orbital region, 3 were from bone, and 1 was from skin. We performed immunohistochemistry on serial sections using our rabbit polyclonal anti–DC-SIGN antibody (manuscript submitted) and mouse monoclonal anti-CDla (Novacastra, Newcastle-upon-Tyne, United Kingdom).

In all 4 specimens of normal skin, the CD1a$^+$ cells in the epidermis and at the dermo-epidermal junction did not express DC-SIGN, whereas CDla$^-$ dendritic cells present within the dermis did (Figure lA-B). In all 8 specimens of LCH, the CDla$^+$ cells within the infiltrate were negative for DC-SIGN, though occasional DC-SIGN–expressing cells could be seen in surrounding tissue acting as a useful internal positive control (Figure 1C-D). Our data are in accordance with previous results suggesting that expression of DC-SIGN and expression of the Langerhans cell–restricted molecule CDla are mutually exclusive (manuscript submitted, Soilleux et al,[4] and Geijtenbeek et al[7]). Our findings, when considered with those of Geissmann et al, indicate that DC-SIGN is only expressed on a subset of dendritic cells with an immature phenotype. Given the very similar expression patterns of Langerin and CD1a,[1] our data suggest that DC-SIGN and Langerin are expressed by mutually exclusive dendritic cell subsets. Whether there are functional differences between Langerhans cells and DC-SIGN$^+$ dendritic cells remains to be determined. The 2 novel

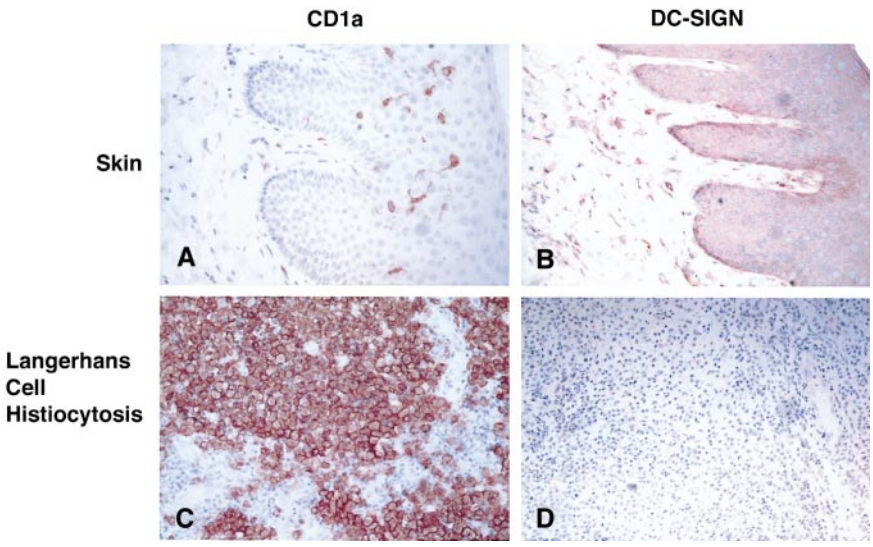

Figure l. DC-SIGN and Langerhans cell histiocyosis. (A) Normal skin shows positive immunostaining for CDla on dendritic cells within the epidermis and at the dermo-epidermal junction. (B) Serial section of skin from panel A shows positive immunostaining for DC-SIGN on cells within the dermis only. No epidermal dendritic cells express DC-SIGN. (C) Langerhans cell histiocyosis from the retro-orbital region showing immunostaining for CDla on numerous large cells. (D) DC-SIGN staining of a serial section of the specimen in panel C shows no expression of DC-SIGN on these large CDla$^+$ cells. Sections were immunostained using the peroxidase technique and counterstained with hematoxylin. Sections were photographed at × 100.

C-type lectins, Langerin and DC-SIGN, may have analogous properties on separate dendritic cell subsets with respect to, for example, their capacity to bind T lymphocytes and lentiviruses.

Elizabeth J. Soilleux and Nicholas Coleman

Correspondence: Elizabeth J. Soilleux, Department of Molecular Histopathology, Addenbrooke's Hospital, Hills Road, Cambridge, CB2 2QQ United Kingdom; e-mail: ejs17@cam.ac.uk

References

1. Geissmann F, Lepelletier Y, Fraitag S, et al. Differentiation of Langerhans cells in Langerhans cell histiocytosis. Blood. 2001;97:1241-1248.
2. Valladeau J, Duvert-Frances V, Pin JJ, et al. The monoclonal antibody DCGM4 recognizes Langerin, a protein specific of Langerhans cells, and is rapidly internalized from the cell surface. Eur J Immunol. 1999;29:2695-2704.
3. Hartgers FC, Figdor CG, Adema GJ. Towards a molecular understanding of dendritic cell immunobiology. Immunol Today. 2000;21:542-545.
4. Soilleux EJ, Barten R, Trowsdale J. DC-SIGN; a related gene, DC-SIGNR; and CD23 form a cluster on 19p13. J Immunol. 2000;165:2937-2942.
5. Geijtenbeek TB, van Vliet SJ, van Duijnhoven GC, Figdor CG, van Kooyk Y. DC-SIGN, a dentritic cell-specific HIV-l receptor present in placenta that infects T cells in trans-A review. Placenta. 2001;22:819-823.
6. Pohlmann S, Baribaud F, Lee B, et al. DC-SIGN interactions with human immunodeficiency virus type 1 and 2 and simian immunodeficiency virus. J Virol. 2001;75:4664-4672.
7. Geijtenbeek TB, Torensma R, van Vliet SJ, et al. Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune responses. Cell. 2000;100:575-585.
8. Geijtenbeek TB, Krooshoop DJ, Bleijs DA, et al. DC-SIGN-ICAM-2 interaction mediates dendritic cell trafficking. Nat Immunol. 2000;1:353-357.
9. Strobl H, Bello-Fernandez C, Riedl E, et al. flt3 ligand in cooperation with transforming growth factor-beta l potentiates in vitro development of Langerhans-type dendritic cells and allows single-cell dendritic cell cluster formation under serum-free conditions. Blood. 1997;90:1425-1434.

To the editor:

Phenotype and genotype expression in pseudohomozygous R2 factor V

Factor Va is the important cofactor for prothrombinase. Factor Va is inactivated by activated protein C (APC) following 3 cleavages of the heavy chain at Arg306, Arg506, and Arg679.[1] Cleavage of normal factor Va by APC at these sites results in the production of M_r 45 000, M_r 30 000, M_r 22 000 and 20 000 fragments.[1] In plasma, following the addition of APC and a synthetic membrane surface (phosphatidylcholine phosphatidylserine [PCPS]), appearance of the M_r 30 000 fragment demonstrates cleavage of normal factor V at Arg306 and Arg506.[2] The M_r 30 000 fragment can be detected in plasma by using an anti–human factor V monoclonal antibody that recognizes an epitope located between residues 307 and 506 of the molecule.[2,3]

The R2 haplotype in factor V is characterized by an A4070G substitution (His1299Arg) in the factor V molecule and is associated with mild APC resistance,[4] but the underlying molecular mechanism remains unclear. We have recently described a thrombotic family with 4 symptomatic members.[5] One of them (I3) was doubly heterozygous for the factor V HR2 haplotype and for the factor V Tyr1702Cys mutation, causing CRM–factor V deficiency. Since the factor V allele predicting the Tyr1702Cys substitution is not expressed at the protein level,[5] the plasma of this patient contains only R2 factor V, in accordance with her reduced factor V levels (FV:Ag 43%; FV:C 36%; normal range 70%-130%) and mild APC resistance (nAPC-sr 0.72, normal values > 0.84). We have concluded that while the His1299Arg substitution in factor V induces APC resistance, the presence of the Tyr1702Cys mutation was responsible for absence of expression of the corresponding allele (previously reported to result in CRM–factor V deficiency[5]). This particular condition (pseudohomozygosity for the factor V HR2 haplotype) offers the opportunity to study the APC-mediated inactivation of R2 factor Va in plasma, which is otherwise possible only in the rare (~0.4% of the general population) homozygous individuals.

Molecular investigations were undertaken in this thrombotic patient (I3, see Castoldi et al[5]). The patient was found to be heterozygous, at both the DNA and messenger RNA (mRNA) levels, for the His1299Arg and Asp2194Gly substitutions, characterizing the HR2 haplotype[4] as well as for the Tyr1702Cys mutation in factor V. No mutation, in addition to the polymorphic