

Epidermal Calprotectin Expression in Lymphocyte-Depleted Cutaneous Graft-versus-Host Reaction

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RESUMEN: Una de las complicaciones más importantes asociadas a los trasplantes de médula ósea es la Reacción Injerto versus Huésped (RIVH). Los regímenes inmunosupresores reducen la severidad de las lesiones cutáneas determinando una forma peculiar de RIVH con depleción linfocitaria (RIVH-DL), caracterizada por pocas células inflamatorias reconocibles y casi una total ausencia de daño epidérmico. Los recientemente actualizados criterios histológicos para la RIVH ayudan poco para el diagnóstico de la RIVH-DL. Como la calprotectina (L1-proteína) ha sido encontrada en diversos tipos de estrés epitelial, analizamos la calprotectina epitelial en la RIVH-DL. La expresión de calprotectina se estudió por inmunohistoquímica usando Mac 387 moAb en 50 casos de RIVH-DL y 40 casos de reacciones tóxicas secundarias a los regímenes condicionantes pre-trasplante o por drogas post-trasplante.

Se detectó calprotectina en queratinocitos de apariencia normal en todos los casos de RIVH-DL y en la mayoría de las dermatitis con citotoxicidad inducidas por drogas. Concluimos que la inmunoreactividad a la calprotectina aparenta ser una clave diagnóstica para la RIVH-DL. Su expresión aparece precozmente en el curso de la RIVH-DL independientemente del grado histológico de la reacción. Sin embargo, no puede ser usada en forma aislada para distinguir entre una RIVH-DL inicial y una dermatitis inducida por drogas.

SUMMARY: One of the most important complications associated with bone marrow transplantation (BMT) is graft-versus-host reaction (GVHR) altering different organs. The immunosuppressive regimen frequently abates the severity of cutaneous lesions to a peculiar lymphocyte-depleted GVHR (LD-GVHR) with scant recognizable inflammatory cells and almost absence of epidermal injury. The recently revisited histological criteria for cutaneous GVHR are of little help in diagnosing such LD-GVHR. As calprotectin (L1-protein) has been reported to be expressed in several types of stressed epithelia, we assessed the epidermal calprotectin expression during LD-GVHR. Calprotectin expression was studied by immuno-histochemistry using the Mac 287 moAb in 50 cases of LD-GVHR and 40 cases of toxic reactions due to the conditioning regimens or to post-transplant drugs. Calprotectin was evidenced in normal looking keratinocytes of all cutaneous LD-GVHR cases and in the vast majority of cytotoxic drug-induced dermatitis. It is concluded that calprotectin immunoreactivity appears to be a diagnostic clue in LD-GVHR. The epidermal calprotectin expression occurs early in GVHR, irrespective of the histological grading. However, it cannot be used alone to distinguish early LD-GVHR from drug-induced dermatitis.

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INTRODUCTION

The early recognition of graft-versus-host reaction (GVHR) after bone marrow transplantation (BMT) is important to initiate prompt therapy and reduce the severity and mortality of the disease. As cutaneous changes are among the earliest clinical signs, a skin biopsy is often performed for diagnostic purpose¹. However, the histologic diagnosis of early cutaneous

GVHR is not always easily reached²⁻⁴. This is specially true when the effective immunosuppressive regimen abates the inflammatory cell infiltrate. In many instances, the skin in a clinically affected area appears histologically unremarkable^{5,6}. Furthermore, the distinction between early cutaneous GVHR and cytotoxic drug-induced dermatitis (CDID) proves to be also difficult, in particular because cytotoxic changes may persist for several weeks following BMT².

The recent reappraisal of the GVHR histological cutaneous grading does not provide diagnostic clues for the lymphocyte-depleted type of GVHR (LD-GVHR), and does not permit to discriminate early cutaneous GVHR from CDID⁷. Various immunohis-

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tochemical markers defining the cell infiltrate⁸⁻¹¹, and the pattern of adhesion molecules¹¹ have been evaluated without establishing a clear cut distinction between early cutaneous GVHR and CDID.

The 365 kDa L1-protein consists of three calcium-non covalently bound polypeptide chains. It is expressed in neutrophilic granulocytes, monocytes, and some reactive macrophages, as well as in mucosal epithelium and reactive epidermis¹²⁻¹⁵. Due to its antimicrobial properties, the name calprotectin was coined for it^{15, 16}. Keratinocytes underlined by inflammatory cells or covering tumors frequently express the L1-protein^{12, 14, 17, 18}, a condition which has been interpreted as a non-specific marker of cellular stress. The L1-protein, persists after formalin fixation and paraffin embedding and is specifically identified using the Mac 387 mAb during routine immunohistochemical processing^{12-15, 17, 18}.

As keratinocytes are among of the major target cells during cutaneous GVHR^{1-4, 6, 19} we were interested to assess the epidermal expression of the L1-protein in cutaneous GVHR. The aim of this work was to compare the epidermal L1-protein expression in 50 LD-GVHR and 40 CDID.

MATERIAL AND METHODS

Fifty cases of LD-GVHR and 40 cases of CDID were retrieved from the dermatopathology department files. Histopathological grading of LD-GVHR was based following Lerner's classification¹. Briefly, grade I displayed vacuolar alterations of the basal keratinocytes. Grade II showed additionally individual necrotic keratinocytes and lymphocytic satellitosis. Grade III corresponded to the formation of discrete blisters secondary to coalescence of apoptotic cells. The studied material included 19 grade I, 21 grade II and 10 grade III LD-GVHR.

Negative immunohistochemical controls consisted in normal skin sections obtained during surgery. Other negative controls on the study material were obtained by omitting the primary antibody in the immunohistochemical procedure. From the formalin-fixed paraffin-embedded material 6 µm thick sections were prepared by routine processing. Immunohistochemistry was performed as follows. Skin sections were pretreated with protease 0.05% for 10 minutes at 37°C. After rinsing, the Mac 387 mouse mAb (Dakopatts, Denmark) was incubated for 15 minutes (1:100 in TBS). Sections were subsequently incubated with biotinylated antimouse link Ig (LSAB + kit prediluted, Dakopatts) at room temperature for 15 minutes. After twice 5-minute TBS rinsing baths, sections were cov-

ered with alkaline phosphatase conjugated streptavidine (LSAB + kit prediluted, Dakopatts) for 15 minutes. New Fuchsin (Dakopatts) was used as chromogen for 5 minutes. Sections were counterstained with hematoxylin and mounted in glycergel (Dakopatts).

RESULTS

A cytoplasmic L1-protein labeling was found in epidermal and follicular keratinocytes of all LD-GVHR cases. The diffuse pattern of L1-protein distribution in the epidermis was frequent, irrespective of the LD-GVHR grades (Fig. 1a). Over 90% of the CDID cases also exhibited epidermal L1-protein expression. The immunostaining was usually more focal than in LD-GVHR (Fig. 1b). The monocyte/macrophage L1-protein expression was present in over 80% of the C-GVHR and CDID.

All control sections gave negative immunoreactivity for the calprotectin in the interadnexal epidermis and no significant background staining.

DISCUSSION

The identification of early cutaneous GVHR can prove to be difficult on skin biopsies²⁻⁴. The widespread use of immunosuppressive regimens in patients receiving bone marrow or peripheral blood stem cell transplantation renders the diagnosis even more difficult due to the lymphocyte-depleted presentation of the lesions. Furthermore, the distinction between early LD-GVHR and CDID is another puzzling problem, especially because cytotoxic changes can persist up to 40 days after drug administration^{2, 20}. The updated histological classification of cutaneous GVHR⁷ neither provide clear cut diagnostic clues for early LD-GVHR nor permit the distinction between early GVHR and CDID. To overcome such shortcomings, the adjunct value of various immunohistochemical cell markers has been studied. The LN-3 mouse mAb, identifying a HLA class II antigen, was found to be expressed in 75% of the cases on endothelial cells in GVHR. It was concluded that LN-3 immunolabeling could help to identify GVHR and discriminate it from CDID⁸. The phenotyping of the epidermal infiltrate²¹, the evaluation of the CD4/CD8 ratio²², the search for the adhesion molecules ICAM-1 and VCAM-1⁹, the TIA-1 Mab for nucleolysin protein identification¹⁰ failed as reliable diagnostic tools for GVHR, although they appeared useful adjunct techniques.

In the present study, L1-protein epidermal expres-

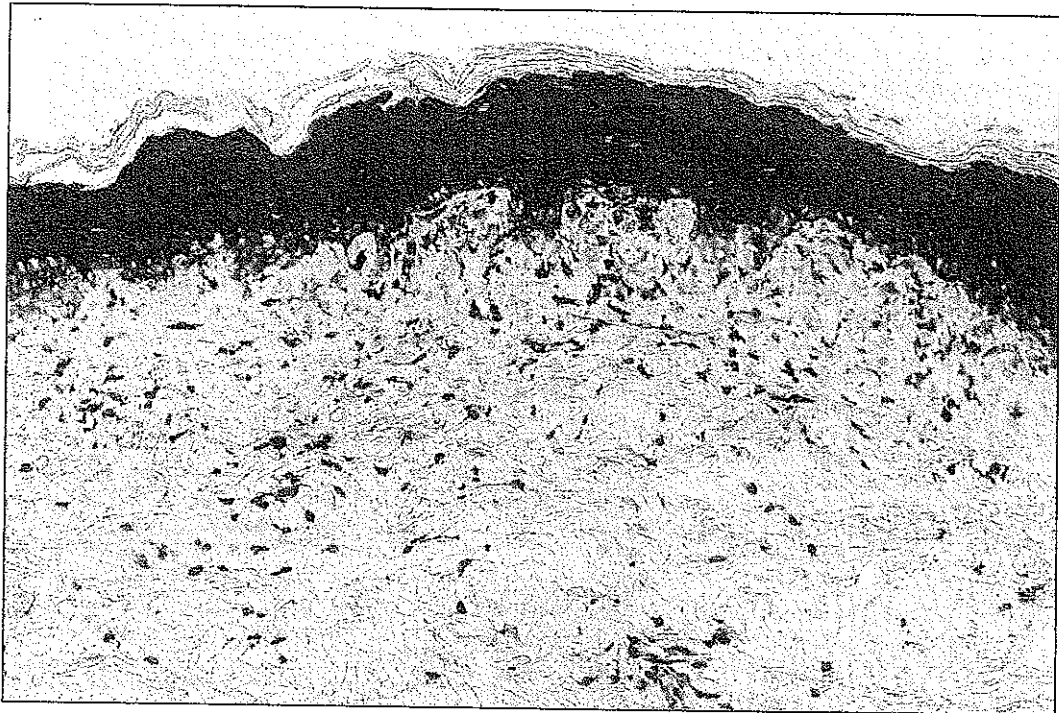
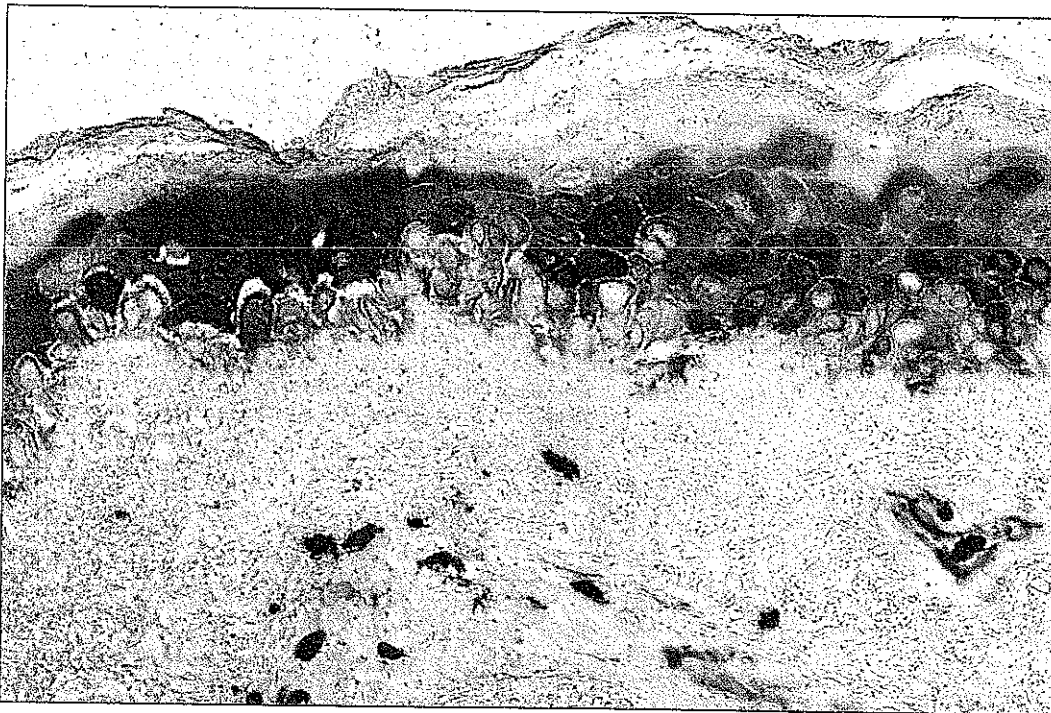


Fig. 1: L1-protein expression in the epidermis using Mac 387 immunostaining.

a: diffuse pattern in LD-GVHR.



b: focal pattern in CDID.

sion was present in all cases of cutaneous LD-GVHR irrespective of the histological grade. The cytoplasmic staining was diffuse all over the epidermal layers even when the dermal inflammatory infiltrate was scant. The L1-protein expression in keratinocytes during LD-GVHR probably discloses the sublethal cell injury. Hence, it appears to be a helpful tool in the event of lymphocyte-depleted rashes featuring a clinical suspi-

cion of GVHR. However, the epidermal L1-protein expression is not specific of GVHR and the immunohistochemical labeling cannot distinguish early LD-GVHR from CDID.

In summary, the L1-protein is expressed in keratinocytes during LD-GVHR. Mac 387 immunolabeling can be easily included during routine immunohistochemical processing and is an interesting

adjunct tool in inflammatory cell-poor histopathological presentations associated with a clinical suspicion of GVHR. However, it is not specific for GVHR and cannot discriminate early LD-GVHR from CDID.

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