Acta Derm Venereol (Stockh) 1997; 77: 184–186

T-cell Receptor Vβ Expression Is Restricted in Dermatitis Herpetiformis Skin

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An infiltrate of T-cells is found in lesional dermatitis herpetiformis skin, but the role of these cells in the pathogenesis of the skin lesions is unknown. The purpose of this study was to examine T-cell receptor Vβ expression in skin biopsies taken from patients with dermatitis herpetiformis. Expression of eleven T-cell receptor Vβ families in biopsies obtained from 10 patients was examined by immunoperoxidase staining and compared simultaneously with peripheral blood lymphocytes. Overrepresentation of Vβ2 ($p<0.02$), Vβ5.2/5.3 ($p<0.01$) and Vβ5.3 ($p<0.05$) was found in lesional dermatitis herpetiformis skin compared with peripheral blood lymphocytes. These results suggest that recognition of an antigen(s) or superantigen is involved in the pathogenesis of dermatitis herpetiformis skin lesions. ***Key words: antigen; superantigen; immunohistochemistry.***

(Accepted October 28, 1996.)

Acta Derm Venereol (Stockh) 1997; 77: 184–186.

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Dermatitis herpetiformis (DH) is an immunobullous disorder associated with gluten sensitive enteropathy (GSE), similar to that found in coeliac disease (1). Both the skin lesions and the enteropathy resolve when gluten is withdrawn from the diet and relapse when gluten is reintroduced (2), suggesting a causal relationship between the ingestion of gluten and the development of the skin and gut changes. A significantly increased number of CD3+ T-cells has been shown to be present in the dermis of lesional DH skin compared with uninvolved skin (3), but the role of these T-cells in the pathogenesis of the skin lesions is unknown. The majority of these T-cells were CD4+ helper T-cells and expressed the CD45RO+ memory antigen and, furthermore, 20–40% were activated. These findings, and the fact that the T-cells were in close apposition with Langerhans' cells in the dermis, suggest an on-going cell-mediated immune response in the skin of patients with DH. However, we have failed to demonstrate a proliferative response by T-cells from lesional DH skin to Frazer's fraction III (the partial peptic tryptic digest of gluten known to be toxic) (4). Moreover, intradermal skin testing with Frazer's fraction III failed to induce delayed type hypersensitivity reactions in DH patients, suggesting that gluten specific T-cells were confined to the intestinal mucosa (5).

Restricted T-cell receptor (TCR) Vβ gene expression would support the hypothesis that recognition of a specific antigen(s) or superantigen is important in the pathogenesis of DH skin lesions. Restricted expression of TCR Vβ genes has been demonstrated in other disorders, such as rheumatoid arthritis (6, 7) and sarcoidosis (8). A recent study from our department demonstrated restriction of the TCR Vβ gene expression in patients with psoriasis (9). The aim of this study, therefore, was to examine the expression of TCR Vβ genes in the skin of patients with DH using monoclonal antibodies and an immunoperoxidase technique.

MATERIAL AND METHODS

Patients and samples

Approval for this study was granted by Kensington, Chelsea and Westminster Health Authority Ethical Committee. Patient consent was also obtained prior to skin biopsies and blood samples being taken.

Ten patients (mean age 54 years, range 27–71) with DH controlled by dapsone and/or sulphonamides were enrolled in the study. Of these patients, 8 were taking a normal diet and 2 a strict gluten free diet (GFD) but had not yet managed to control their rash with diet alone. Patients were asked to discontinue their medication and two 4-mm punch biopsies were taken 7 days later: one from a blister and the other from uninvolved skin, a few centimetres from the involved site. Skin biopsies were snap frozen in OCT (Tissue Tek©) and stored in liquid nitrogen. Six-micrometre sections were cut on a cryostat (Slee, London) and stored at $-80°C$ until staining.

Peripheral blood lymphocytes (PBL) were isolated from 20 ml of heparinised blood by Ficoll-Hypaque density gradient centrifugation.

Antibodies

Monoclonal antibodies, which have been shown to recognise specifically a variable region epitope on the β chain of the following Vβ families were used: Vβ2, Vβ8 (both kindly donated by Professor Boylston, University of Leeds), Vβ3 (a gift kindly donated by Dr. M. Owen, ICRF (10)), Vβ5.1, Vβ5.2/5.3, Vβ5.3, Vβ6.7, Vβ12.1, Vβ13.1/13.3 (T-Cell Sciences Inc., Cambridge, MA, U.S.A.), Vβ2, Vβ9 and Vβ17 (Immunotech, Marseille, France). Other antibodies used were pan αβ (T cell Sciences Inc., Cambridge, MA, U.S.A.) and CD3 (Becton Dickinson, Oxford, U.K.).

Immunoperoxidase staining

The avidin-biotin peroxidase technique, using a Vectastain Elite ABC kit (Vector Laboratories, Peterborough, U.K.), was used to stain the skin sections. Briefly, the sections were air-dried, fixed in chloroform:acetone (50:50) and blocked with normal serum. Endogenous peroxidase was quenched with 0.3% H_2O_2 in methanol. The sections were then incubated with primary mouse monoclonal antibodies (all primary antibodies were used neat except Vβ9 and Vβ17, which were used at dilutions of 1:10 and 1:5, respectively), followed by biotinylated horse antimouse secondary antibody at a dilution of 1:200 and then avidin-biotin peroxidase complex. The colour reaction was developed with the substrate 3-amino-9-ethyl carbazole (AEC; Sigma, Poole, Dorset, U.K.), and the sections were counterstained with haematoxylin and mounted in glycergel (Dako, High Wycombe, Bucks, U.K.). Control sections included substitution of the primary antibody with a mouse IgG isotype control antibody (Vector Laboratories, Peterborough, U.K.) and omission of the primary antibody.

Quantification

The slides were coded, and positively stained cells in four to six consecutive high-power fields (×400) in two sections were counted in a blind manner.

Flow cytometric analysis

Flow cytometric analysis was performed on PBL obtained from the DH patients at the time of biopsy. PBL were separately incubated with each anti-TCR Vβ antibody (all used neat with the exception of pan αβ, Vβ9 and Vβ13.1/13.3 antibodies, which were used at a dilution of 1:2) and followed by FITC-labelled sheep anti-mouse IgG antibody

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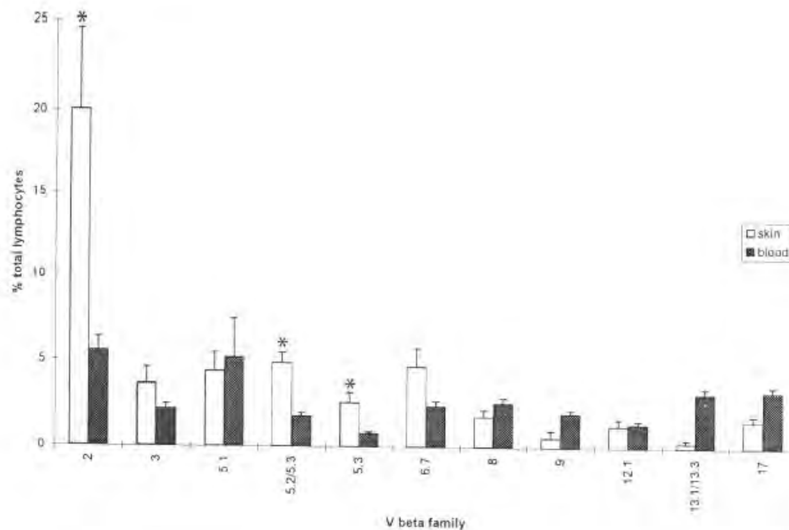

Fig. 1. Expression of Vβ families in the lesional skin □ and blood ■ of 10 patients with dermatitis herpetiformis, expressed as a mean percentage of CD3+ T-lymphocytes. * indicates Vβ families significantly elevated in the skin compared with peripheral blood lymphocytes using Wilcoxon's paired rank sum test ($p<0.05$).

(Sigma), diluted 1:10 and double-stained for CD3 expression with phycoerythrin-conjugated Leu 4 antibody (Becton-Dickinson), and then analysed in an EPICS flow cytometer (Coulter Electronics Ltd.). FITC-labelled goat anti-mouse IgM (Sigma), diluted 1:100, was used with the Immunotech Vβ2 antibody.

Statistics

The results are expressed as mean ± SEM: expression of Vβ families in lesional skin and blood was expressed as a mean percentage of CD3+ T-lymphocytes. The Mann-Whitney *U*-test was used to compare the T-cell counts in involved skin with those in uninvolved skin. Comparison between Vβ TCR expression in the skin and peripheral blood was made using Wilcoxon's paired rank sum test.

RESULTS

Expression of Vβ families in involved skin compared with PBLs

The number of CD3-positive lymphocytes was significantly increased in the upper dermis of involved skin (89 ± 15 per high-power field), compared with uninvolved skin (17 ± 3 per high-power field) ($p<0.005$). No CD3-positive lymphocytes were observed in the epidermis.

T-lymphocytes expressing each Vβ family in the dermis of involved skin and PBLs are represented as mean values ± standard error of the mean (SEM) for the group as a whole in Fig. 1; Wilcoxon's paired rank sum test was used to analyse the data. For each individual patient, over-representation of specific Vβ receptors was considered significant if the number of T-cells expressing the Vβ receptor in the skin was at least double the number present in the PBLs. Thus, we found that 6 out of 10 patients had significant over-representation of Vβ2 (20.1 (mean) ± 4.5 (SEM) in skin; 5.6 (mean) ± 0.8 (SEM) in blood, $p<0.02$); 8 out of 10 patients had over-representation of Vβ5.2/5.3 (4.9 ± 0.5 in skin; 2 ± 0.2 in blood, $p<0.01$); and 7 out of 10 patients had over-representation of Vβ5.3 (2.6 ± 0.6 in skin; 0.8 ± 0.1 in blood, $p<0.05$). Much larger numbers of cells expressing TCR Vβ2 were seen in comparison with TCR Vβ5.2/5.3 and Vβ5.3.

Furthermore, 5 out of 10 patients demonstrated cytoplasmic staining with anti-Vβ5.3 antibody in involved but not uninvolved skin. These cells were scattered throughout the dermis and some were present in the vesicles. Two of these patients demonstrated similar staining with anti-Vβ6.7 antibody. These cells were not counted when sections were being evaluated, because they did not exhibit the expected membrane staining as observed in other positively staining cells.

In an attempt to confirm the increased Vβ2 expression in DH skin shown by A. Boylston's antibody, a second Vβ2 antibody (Immunotech, Marseille, France) was employed. Similar results were obtained with the two antibodies for the PBLs, although the Immunotech antibody stained approximately 1% less cells. Unfortunately, the Immunotech antibody gave high background staining on tissue sections, which precluded enumeration of positively staining cells.

Interpretation of staining with the Vβ antibodies was not possible, as only small numbers of T-cells were found in uninvolved skin.

DISCUSSION

This study has shown over-representation of specific TCR Vβ subsets, namely Vβ2, Vβ5.2/5.3, Vβ5.3, in DH lesional skin compared with blood. Large numbers of T-cells expressing Vβ2 were observed in DH lesional skin (Fig. 1) compared with Vβ5.2/5.3 and 5.3. Indeed, in one patient, 50% of the infiltrating T-cells expressed Vβ2. We have previously also shown preferential usage of TCR Vβ2 in psoriasis by immunohistochemistry (9), a finding which has been confirmed by Leung et al. (11). It could be hypothesized that T-cells bearing TCR Vβ2 "home in" to the skin in inflammatory dermatoses. However, Leung et al. (11), using the same Vβ2 antibody and a similar immunohistochemical technique as we did, demonstrated that expression of TCR Vβ2 was not significantly increased in atopic eczema or irritant dermatitis. Nor was TCR Vβ2 expression found to be increased in lichen planus by PCR (12). With regard to the TCR repertoire in normal skin, conflicting results have been obtained: one study demonstrated overexpression of TCR Vβ2 in normal skin by PCR, although this was found to be polyclonal by sequence analysis (13); another study demonstrated increased expression of TCR Vβ1, Vβ7, Vβ14 and Vβ16 by PCR (12); and more recently, TCR Vβ3 and Vβ14 were shown to be significantly overexpressed in normal breast skin compared to PBLs (14).

DH is strongly associated with HLA A1, B8, DR3, DQ2,

with over 95% of DH patients expressing the DQ2 antigen (15). There is now good evidence from twin and family studies that the human TCR repertoire is primarily genetically determined (16). Moreover, family studies support HLA class I and class II antigens as having a profound effect on the selection of T-cells expressing particular Vβ segments (17). It has been proposed that HLA associations with disease may be explained by the effects of HLA antigens on determination of the TCR V segment frequencies, thereby biasing the individual toward the development of autoimmunity (17). It is therefore perhaps not surprising that we found over-representation of certain Vβ families in DH lesional skin.

We have previously hypothesized that the skin lesions of DH are due to T-cell mediated responses directed against an as yet unidentified autoantigen present in the skin (4). Over 90% of patients with DH have evidence of a GSE (18,19). It is highly probable that the presence of GSE is necessary for the development of skin lesions in these patients. We have previously proposed that damage to the small bowel by gluten exposes an antigenic determinant in the small intestine, which is similar to a corresponding epitope in the skin (4). T-cells recognising this antigen would therefore migrate from the small intestine to the skin. One candidate antigen could be reticulin; patients with DH have anti-reticulin antibodies (20), and furthermore the titre of antibody correlates with the degree of intestinal damage (21). We are currently investigating this possibility.

An intriguing finding from this study was the presence of cytoplasmic staining of Vβ5.3 in 5 out of 10 patients and of Vβ6.7 in 2 out of 10 patients. It is known that specific recognition of antigen by T-cells is followed by internalisation of the TCR (22). It is possible, therefore, that these cells represented recently activated T-cells, in which surface re-expression of the TCR had not yet occurred. Unfortunately, we have been unable to prove this.

To date, there have been no published studies on TCR Vβ expression in the small intestine of patients with DH. Troncone et al. (23) have demonstrated over-representation of TCR Vβ8 by immunohistochemistry in the lamina propria of untreated coeliac patients compared with controls. These findings, they concluded, supported the role of either an antigen or superantigen in the pathogenesis of coeliac disease. It remains to be shown whether there is over-expression of specific TCR Vβ(s) in the small intestine of DH patients.

In summary, this study has shown bias of usage of TCR Vβ receptors, which implicates the involvement of an antigen or superantigen in the pathogenesis of DH skin lesions.

ACKNOWLEDGEMENT

This study was funded in part by a grant received from St. Mary's Hospital Joint Standing Research Committee.

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