Early or Late Expression of Ia Antigen on Mouse Keratinocytes in Allergic Contact Dermatitis?

Sir,
Recent progress has been made in our understanding of the pathogenesis of allergic contact dermatitis (ACD). However, the cells able to present hapten during the elicitation of ACD are not clearly defined. The role of HLA-DR<sup>+</sup> keratinocytes as antigen-presenting cells in the efferent limb of hapten immune response is still debated. Kinetic studies have reported either early or late induction of MHC class II molecule expression by keratinocytes after hapten elicitation (1–3). We would like here to report on personal observations in oxazolone-induced ACD in Balb/c mice, showing that Ia antigen expression on keratinocytes represents an early event in the course of ACD.

Mice were sensitized on the abdomen with 50 μl of a solution containing 2% oxazolone (4-ethylmethylen-2-phenylazo-red) in acetone/olive oil. Five days later, animals were painted once on both faces of the left ear with 25 μl of 0.4% oxazolone. The right ear received the vehicle alone. Control mice received vehicle alone for the sensitization and 25 μl of 0.4% oxazolone for the elicitation. The delayed-type hypersensitivity response was assessed on days 1, 2, 3 and 7 by the mouse ear swelling test (4) (Fig. 1). Some mice were killed 12 h, 24 h, 48 h, 72 h and 7 days after the elicitation, and their ears were immediately frozen in liquid nitrogen. Ia expression was assessed on cryostat sections of the various skin samples using a rat monoclonal antibody (CD311), specific for mouse Ia antigen, kindly provided by Dr. A. L. Glasebrook (Eli Lilly, Indianapolis), and an immunoperoxidase technique, CD311, a monomorphic anti-Ia monoclonal antibody (MoAb), recognizes a determinant present on MHC class II molecules of a large number of H-2 haplotypes including d, k and b (5, 6). Foci of I<sup>+</sup> keratinocytes could be observed as early as 12 h after elicitation of contact sensitivity to oxazolone (Fig. 2). Maximum expression was seen at 48 h with a continuous staining of the basal cell layer, and basal keratinocytes were still I<sup>+</sup> on day 7. Only weak and focal labelling of keratinocytes was noted at 48 h in oxazolone-challenged skin in the non-sensitized animals.

Our results, demonstrating that Ia antigen expression by keratinocytes is an early event in ACD, are in accordance with the study of Roberts et al., in which oxazolone-sensitized mice expressed at 24 h a diffuse or a focal I<sup>+</sup> staining of the basal keratinocytes (1). In humans, I<sup>+</sup> keratinocytes were found in ACD as early as 6 h after elicitation (2). However, early I<sup>+</sup> molecule expression on keratinocytes was not found by Stringer et al. (3), who reported that I<sup>+</sup> keratinocytes could only be detected 3 days after elicitation of contact sensitivity using oxazolone in Balb/c mice.

One possibility, which might explain these discrepancies,

![Fig. 1. Contact hypersensitivity response in oxazolone- (black bars) or vehicle- (white bars) sensitized Balb/c mice. Groups of Balb/c mice (six animals per group) were sensitized on the abdomen (50 μl) with either 2% oxazolone or vehicle alone. All animals were challenged on the left ear with oxazolone (25 μl of 0.4% solution) and on the right ear with vehicle. Ear swelling was measured at different times after challenge. It was calculated by subtracting the pre-challenge value from the post-challenge one and then further subtracting any swelling recorded for the vehicle-challenged ear (never more than 10%) from the swelling recorded for the antigen-challenged ear. Bars represent the mean ± SEM units of ear swelling in each group.](image1.png)

![Fig. 2. Ia antigen expression on a cryostat skin section of oxazolone-challenged mouse ear 12 h after challenge (indirect immunoperoxidase staining; magnification x40). Foci of Ia-positive keratinocytes (*) are observed on both sides of the ear. C, ear cartilage.](image2.png)
may relate to the type of anti-IL antibody used. We hypothesize that during the inflammatory reaction occurring after hapten application, the density of Ia molecules progressively increases with time and that early after elicitation keratinocytes express a low density of Ia antigens which could be under the sensitivity threshold of some but not all anti-Ia antibodies. The precise role of Ia+ keratinocytes in the initiation of the ACD reaction remains to be clarified.

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Immunohistochemical Screening of Neuropeptides in Cutaneous Macular Lesions of Leprosy

Sir,

Leprosy peripheral neuropathy is caused by an inflammatory involvement of peripheral nerves; however, the interaction of mononuclear leukocytes and the neural fibres as well as the functional molecular disturbances that take place in nerves affected by the disease remain to be determined. The first study of neuropeptide expression in the bipolar spectrum of leprosy was carried out by Karanth et al. (1), who could detect significant alterations of calcitonin gene-related peptide (CGRP), neuropeptide tyrosine (NPY), vasoactive intestinal polypeptide (VIP), substance P (SP) and protein gene product (PGP) 9.5 immunoreactivity in lepromatous, tuberculoid and indeterminate leprosy patients.

We also think that immunohistochemical screening of early leprosy cutaneous macular lesions for changes of neuropeptide expression may contribute to the understanding of initial leprosy nerve alterations.

Therefore, we studied the cutaneous macular lesions of 5 patients using the indirect immunofluorescence technique for detecting abnormalities in the neural fibre immunoreactivity for somatostatin, methionine-enkephalin, α, β- and γ-melanocyte stimulating hormone (MSH), peptide histidine isoleucine amide (PHI), growth-associated protein (GAP) 43, and galanin. Skin punch biopsies (5 mm) were obtained under local anaesthesia (Xylcocain/adrenalin, 20 mg/ml+12.5 μg/ml). Age-, sex-, and body region-matched pieces of skin from normal individuals were taken as controls.

Our study did not show any difference between pathological and normal skin regarding the expression of these neuropeptides, which is in contrast to the ones investigated by Karanth et al. (1).

Alteration of MSH immunoreactivity associated with leprosy pigmentation disorders, as well as disturbances in the production of additional neuropeptides, would be likely to occur in early leprosy; however, immunohistochemical methods may not be sensitive enough to detect early modifications of these neuropeptides. Thus, our results must be interpreted with some caution.

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Sergio Luiz Gomes Antunes 1–3, Euzenir Nunes Sarno 4, Guinilla Holmivik 5 and Olof Johansson 5, 6. "Experimental Dermatology Unit, Department of Neuroscience, Karolinska Institute, S-171 77 Stockholm, Sweden, and Department of Leprosy, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil.

* For offprint request

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