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 haptens during the elicitation of ACD are not clearly defined. The role of HLA-DR$^+$ 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-ethoxymethylene-2-phenyloxazol-5-one) 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 Ia$^+$ 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 Ia$^+$ on day 7. Only weak and focal labelling of keratinocytes was noted at 48 h in oxazolone-challenged skin in the non-sensitized animals.

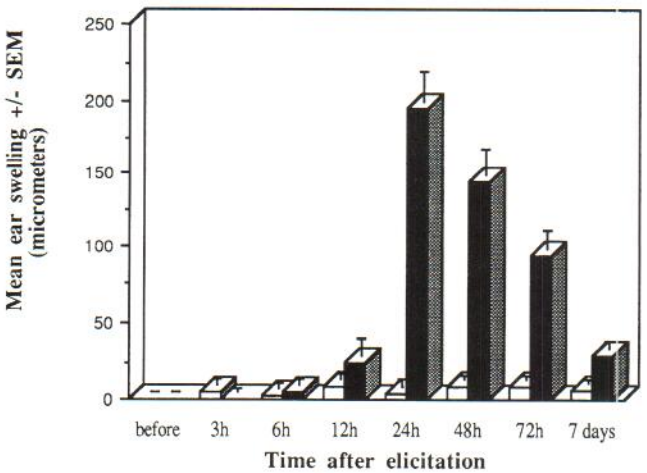

Fig. 1. Contact hypersensitivity response in oxazolone- (*black bars*) or vehicle- (*white bars*) sensitized Balb/c mice. Groups of Balb/c mice (six animals per group) were sensitized on the abdomen (50 µl) with either 2% oxazolone or vehicle alone. All animals were challenged on the left ear with oxazolone (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.

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 Ia$^+$ staining of the basal keratinocytes (1). In humans, Ia$^+$ keratinocytes were found in ACD as early as 6 h after elicitation (2). However, early Ia molecule expression on keratinocytes was not found by Stringer et al. (3), who reported that Ia$^+$ 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,

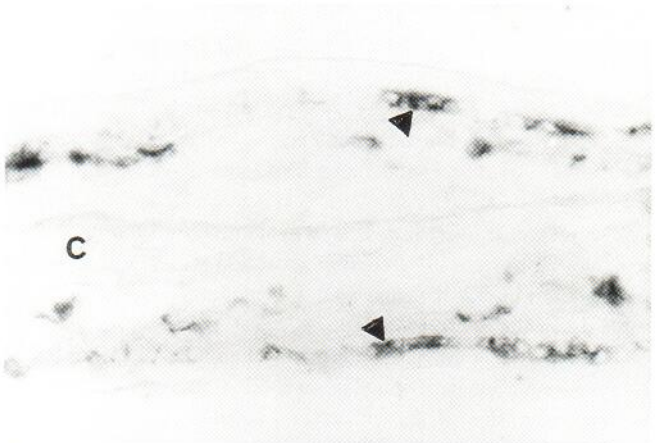

Fig. 2. Ia antigen expression on a cryostat skin section of oxazolone-challenged mouse ear 12 h after challenge (indirect immunoperoxidase staining; magnification ×40). Foci of Ia-positive keratinocytes (▶) are observed on both sides of the ear. *C*, ear cartilage.

may relate to the type of anti-Ia 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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