Contact Allergens Modulate the Expression of MHC Class II Molecules on Murine Epidermal Langerhans Cells by Endocytotic Mechanisms

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MHC class II molecules play an important role during the sensitization phase of allergic contact dermatitis. To study the influence of contact allergens on the expression of these molecules by murine epidermal Langerhans cells (LC), we performed a flow-cytofluorometric analysis of the Ia-antigen expression after in vivo application of contact allergens. A distinct decrease in the Ia-antigen expression of the entire LC population was noticed 3 h after in vivo application of the contact allergen 2,4-dinitrofluorobenzene (DNFB). This decrease was transient and balanced 24 h after in vivo application of DNFB. A downregulation was also detectable after in vivo application of the contact allergens 1-chloro-2,4-dinitrobenzene (DNCB), oxazolone, $K_2Cr_2O_7$, 2,4,6-trinitrochlorobenzene (TNCB), and toxic concentrations of the irritant compound sodium dodecyl sulfate (SDS). In vitro studies showed that freshly prepared as well as 3-d cultured LC downregulated their Ia-antigen expression in the presence of DNFB, which was used as a model compound. This decrease was not inhibited by the MHC class II molecule transport-inhibitor brefeldin A nor by the ionophore monensin. The inhibition of receptor-mediated endocytosis with hypertonic media (0.45 M sucrose) abolished the DNFB-mediated downregulation of Ia-antigen expression. An accelerated clearance of cell-surface–expressed antibody-labeled IA molecules was detectable in the presence of DNFB. Internalization studies carried out with peroxidase-labeled anti-IA–antibody complexes showed remarkable alterations in the intracellular distribution of endocytosed material under the influence of subtoxic concentrations of DNFB, DNCB, $K_2Cr_2O_7$, and TNCB. The irritant substance sodium dodecyl sulfate (SDS) influenced the intracellular distribution pattern of internalized material only when used in toxic concentrations. An augmented participation of MHC class II molecules in endocytotic processes is mediated by reactive substances like contact allergens and might contribute to the processing and presentation of these compounds. *J Invest Dermatol 98:700–705, 1992*

MHC class II molecules play an important role during the initiation and elicitation of a T-cell–mediated immune response like allergic contact dermatitis. The expression of class II molecules by freshly prepared as well as in vitro cultured murine Langerhans cells (LC) has been studied in detail [1,2]. In contrast to the cell membrane expression of Ia-antigens, which increased severalfold, a dramatic decrease in biosynthesis of Ia-anti-

gens was noticed for in vitro cultured LC [3–5]. These cultured LC are thought to resemble in vivo activated LC [6–8]. Recently, Aiba and Katz [9] examined the in vivo activation of murine LC under the influence of contact allergens. A remarkable increase in Ia-antigen expression, as well as in the functional capabilities of LC, was found after in vivo application for 24 h of different contact allergens.

Endocytosis of class II molecules has been described to be a capability of freshly prepared and, to a minor degree, cultured human LC [10,11]. Furthermore, an augmentation of endocytotic subcellular structures was found for murine LC during the first hours after in vivo application of contact allergens [12].

In this report we focused on the first hours of sensitization to contact allergens and examined whether MHC class II molecules are involved in the endocytosis of cell membrane constituents under the influence of contact allergens. We found a reduced expression of MHC class II molecules at the cell surface of in vivo and in vitro haptenized LC, as well as after in vivo treatment with toxic amounts of the irritant compound sodium dodecyl sulfate (SDS). Evidence is presented that endocytotic mechanisms account for this decrease under the influence of contact allergens. The possible implications for the processing and presentation of contact allergens will be discussed.

MATERIALS AND METHODS

Animals Eight- to twelve-week-old Balb/c mice of both sexes were obtained from the animal facilities of the Department of Dermatology, University of Mainz, Germany. Breeding stock was origi-

nally supplied by the Zentralinstitut für Versuchstiere, Hannover, Germany.

Antibodies Mouse MoAb MK-D6 (anti-I-A^d [13]) and 14-4-4S (anti-I-E^{k,d} [14]), as well as rat MoAb M1/9.3.4.HL.2 (anti-CD45 antigen [15]) were obtained from the American Type Culture Collection, Rockville, MD, and used as culture SN containing more than 5 μg/ml antigen-specific IgG. FITC-labeled goat anti-mouse IgG and peroxidase-labeled sheep anti-mouse IgG antibodies were from Sigma, St. Louis, MO. R-phycoerythrin (PE)-labeled goat anti-rat IgG antibodies were obtained from Southern Biotechnology, Birmingham, AL.

Contact Allergens and Irritants The contact allergens 2, 4-dinitrofluorobenzene (DNFB), 1-chloro-2,4-dinitrobenzene (DNCB), and 4-ethoxymethylene-2-phenyl-oxazol-5-one (oxazolone) were from Sigma; 2,4,6-trinitrochlorobenzene (TNCB) was purchased from Polyscience, Inc., Warrington, PA. All these substances were dissolved in acetone/olive oil (4 : 1) for in vivo experiments and in acetone for in vitro experiments. $K_2Cr_2O_7$ was supplied by Merck, Darmstadt, Germany; the irritant SDS was purchased from Sigma; both substances were dissolved in aqua for in vivo and in vitro experiments.

Chemical Treatment Ears of Balb/c mice were painted on each side with 17 μl of DNFB (0.3% v/v), DNCB (1% w/v), oxazolone (3% w/v), TNCB (1% w/v), $K_2Cr_2O_7$ (1% w/v), and SDS (1%, 5%, 10% w/v), respectively, or the corresponding solvent. After 3 h, and in some experiments with DNFB after 24 h, the mice were killed and their ears collected. In addition, ears and pelts of untreated mice were prepared. Epidermal cell suspensions from haptenized and untreated epidermis were obtained by the procedure outlined recently [5] and were immediately subjected to flow cytometric analysis or short-term culture.

Epidermal Cell Culture Bulk epidermal cells ([EC], 10^6/ml) obtained from untreated mice were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) heat-inactivated fetal calf serum (FCS) (Gibco, Grand Island, NY) 2 mM L-glutamine, and 100 IU/ml penicillin/100 μg/ml streptomycin in polystyrene Petri dishes for up to 3 h at 37°C in the presence of DNFB (final concentration in medium 5 μg/ml) or 0.1% acetone as solvent control. In some experiments, 1 μg/ml brefeldin A (Sigma), 10 μM monensin (Sigma), or 0.45 M sucrose were added. EC precultured for 3 d under the conditions outlined above were also short-term cultured for 3 h in the presence or absence of DNFB. After short-term culture, EC were harvested and subjected to FCM analysis.

Flow Cytometric Analysis 10^6 cells were fixed in 500 μl of 2% paraformaldehyde (PFA) for 10 min at RT. Subsequently, 1 ml of ice-cold phosphate-buffered saline (PBS)/2% horse serum ([HS], Gibco) was added and the cells were spun down in the cold for 5 min at 380 × g. This washing procedure was repeated once, followed by the addition of each 250 μl supernatant of a MoAb recognizing Ia- and CD45-antigen, respectively. Cells were incubated for 15 min at 4°C and washed again as outlined above. Second-step antibodies were diluted in PBS/2% HS and added at final concentrations of 7.5 μg/ml for fluorescein-isothiocyanate (FITC) and 5 μg/ml for PE-labeled reagents. The cells were kept again at 4°C for 15 min and washed as outlined, followed by analysis on a FACScan (Becton-Dickinson, Mountain View, CA). The mean fluorescence intensity of 3000 Ia and CD45 double-positive cells was determined by software gating and expressed as percent of the mean fluorescence intensity of freshly prepared untreated LC. In selected experiments, unfixed cells were stained with MoAb against Ia-antigen and FITC-labeled second-step antibodies according to the described procedure, and, finally, 1 μg/ml propidium iodide ([PI], Sigma) was added prior to FACScan analysis to exclude dead cells from analysis by software gating.

Internalization Studies with MoAb-Labeled Ia-Antigen EC precultured for 1 d were stained with MoAb MK-D6 for 15 min at
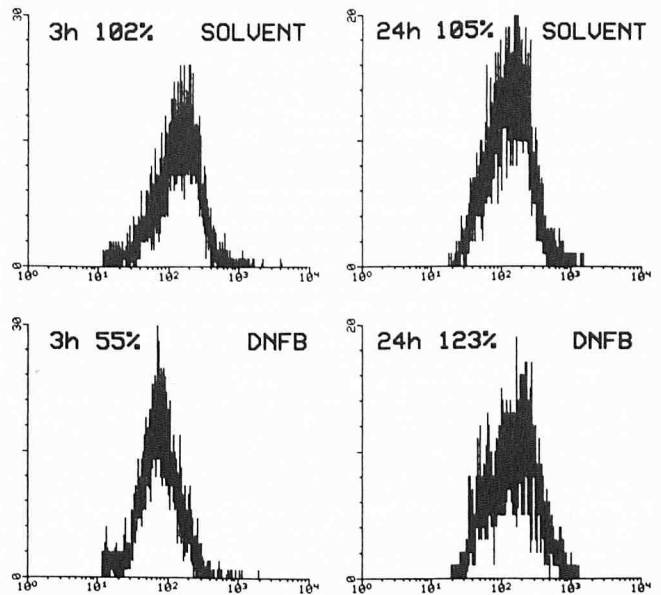

Figure 1. The downregulation of IA molecule expression during the first hours after DNFB application is transient and balanced after 24 h. The histograms show the representative fluorescence intensity for the IA-antigen expression of LC 3 and 24 h after in vivo haptenization with 0.3% DNFB and acetone/olive oil as solvent. The mean fluorescence intensity is calculated as percent of untreated cells. The histograms are representative for four independent experiments.

4°C, washed twice in ice-cold PBS/2% HS, and cultured at 37°C for 1 and 3 h in the presence of 5 μg/ml DNFB or 0.1% acetone as solvent. After fixation with PFA, the remaining cell-surface–associated MoAb was detected by staining with FITC-labeled second-step antibody and subsequent flow-cytometric analysis. To demonstrate the distribution pattern of the internalized MoAb-IA complexes precultured EC were labeled with MoAb MK-D6 and peroxidase-coupled second-step antibody, washed twice, and cultured for 1 h with or without different concentrations of DNFB, DNCB, TNCB, $K_2Cr_2O_7$, SDS, or solvent. Remaining cell-surface–associated antibodies were removed by an acid wash in 0.5 M NaCl/0.2 M acetic acid, ph 2.5, for 5 min at 4°C [16,17] and cells were washed once in PBS/HS. To visualize the intracellular peroxidase activity, cytospin preparations from acid-treated and untreated cells were incubated in AEC solution (3-amino-9-ethylcarbazol in dimethylformamide) as described in [5] and mounted for light microscopy. Appropriate controls carried out with freshly labeled cells were used to assess the efficiency of the acid wash procedure. To facilitate the documentation, a pre-enrichment of LC by an indirect panning method with MoAb MK-D6 was performed in some experiments as outlined previously [5]. To monitor the viability of haptenized cells at the end of the short-term culture, the same experiments were performed with MoAb MK-D6 and FITC-labeled second-step antibodies. The ratio between viable and dead LC was monitored by staining with PI and subsequent FACScan analysis.

RESULTS

Decrease of Ia Antigen Expression on LC after In Vivo Application of Contact Allergens The in vivo application of 0.3% DNFB for 3 h resulted in a uniform reduction of Ia antigen expression of the entire LC population (Fig 1). This decrease was balanced after 24 h of continued DNFB exposure and, in addition, a slight increase in IA molecule expression was detectable in comparison to the corresponding control (Fig 1). No significant changes in the forward or sideward scatter were noticed between untreated, solvent-treated, and DNFB-treated cells (not shown), excluding
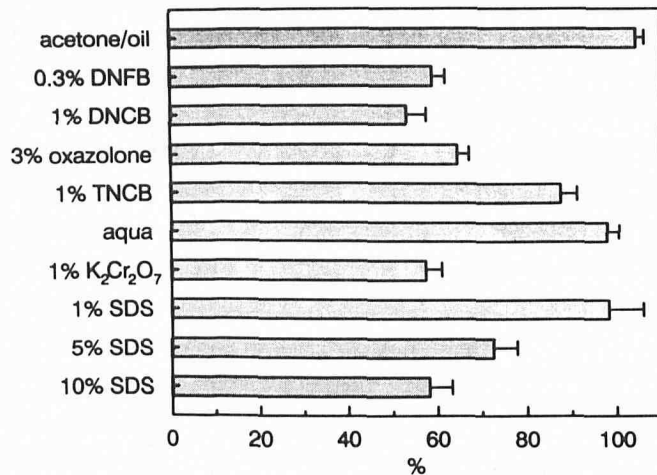
Figure 2. Reduced IA molecule expression of LC after in vivo treatment with various contact allergens and subtoxic (1%) as well as toxic (5%, 10%) concentrations of SDS. The relative IA molecule expression in percent of untreated cells 3 h after application of the indicated compounds is shown. Mean and SEM of five experiments are given.

that differences in fluorescence intensity might result from cell shape changes under the influence of DNFB.

We focused on this early downregulation of IA molecule expression and examined whether other contact allergens like DNCB, oxazolone, TNCB, and $K_2Cr_2O_7$ would also reduce the expression of these molecules by LC during the first 3 h as noticed for DNFB. Although pronounced differences were seen with regard to the degree of the downregulation, all haptens caused a distinct reduction of IA molecule expression by LC in comparison to untreated cells, which were defined as 100% and closely resembled solvent-treated cells (Fig 2). A comparable reduction was noticed for the expression of IE and CD45 molecules (not shown). In pilot experiments with DNFB an in vivo application for 3 h was found to be optimal to detect the described downregulation of Ia-antigen. To address the question of whether contact allergens influence the expression of Ia-antigen by toxic mechanisms, we included controls with different concentrations of the toxic compound SDS (Fig 2). Subtoxic amounts of SDS (1%) did not influence the IA molecule expression, whereas 5% and more pronounced 10% SDS resulted in a distinct reduction of LC viability (down to 42% after treatment with 10% SDS when compared to untreated cells) and also in depressed expression of IA (Fig 2), as well as IE and CD 45 molecules (not shown). In general, there was a strict correlation between toxicity and downregulation of Ia-antigen expression for this inducer of toxic contact dermatitis [18]. Although in some experiments a 5–25% lower viability of LC was detectable after treatment with contact allergens, no correlation between downregulation of Ia-antigen expression and toxicity was detectable. A strong decrease in the expression of these molecules could be seen without any toxic effects. In representative experiments dead cells were excluded from analysis by PI staining of unfixed cells with essentially the same results, as indicated in Fig 2.

Modulation of Ia-Antigen Expression on LC During In Vitro Culture with DNFB Subsequent in vitro experiments with DNFB were undertaken to study the mechanism of the observed modulation of Ia-antigen expression under the influence of contact allergens. Freshly prepared EC were cultured in the presence or absence of DNFB and were compared to EC precultured for 3 d. From pilot experiments a final concentration of 5 μg/ml DNFB and an incubation time of 3 h was found to be optimal. Whereas fresh LC upregulated their IA molecule expression in the presence of solvent, a distinct decrease was seen under the influence of DNFB during short term culture (Fig 3). Continuous in vitro culture for 3 d

of bulk EC preparations resulted in upregulated expression of IA molecules, which did not change during the short-term culture for 3 h in the presence of solvent but dramatically decreased during treatment with DNFB (Fig 3). This diminished IA molecule expression of both fresh prepared as well as cultured LC was strictly related to viable cells, as evidenced by experiments with PI staining of unfixed EC (not shown). Again the expression of IE and CD45 molecules was strongly correlated to the observed decrease of IA molecule expression. In some experiments a reduced viability of LC (down to 50% of the solvent control) was noticed in the presence of 5 μg/ml DNFB for preparations with a low starting viability of 75–90% of the entire EC population, which routinely exceeded 90%. A strictly subtoxic concentration like 1 μg/ml DNFB showed a suboptimal but still significant influence on Ia-antigen expression of LC (not shown).

Influence of Brefeldin A, Monensin, and Hypertonic Media on the Decrease of Ia-Antigen Expression To gain insight into the mechanism of the observed downregulation of Ia-antigen expression by LC, we used brefeldin A to block the transport of newly synthesized class II molecules to the cell membrane [19,20]. Brefeldin A was able to inhibit the upregulation of IA expression during short-term culture in the presence of solvent but failed to prevent the decrease under the influence of DNFB (Fig 4A). Comparable results were obtained with the ionophore monensin (Fig 4B), which has been reported to interrupt not only the transport of newly synthesized but also of recycled class II molecules [17,21]. Furthermore, we cultured EC in the presence of hypertonic media (by addition of 0.45 M sucrose), which results in an inhibition of receptor-mediated endocytosis [22]. This treatment did not only block the upregulation of IA molecules by solvent-treated cells, but also the downregulation in the presence of DNFB (Fig 4C). For all three sets of experiments the same results could be obtained after exclusion of dead cells with PI.

Internalization of Antibody-Labeled MHC Class II Molecules in the Presence of Contact Allergens To demonstrate that contact allergens augment the internalization of cell-surface–expressed MHC class II molecules we examined the clearance of MoAb-labeled cell-surface–expressed IA molecules under the influence of DNFB in comparison to solvent-treated cells. As shown in Fig 5, an accelerated decrease of cell-surface–associated MoAb was
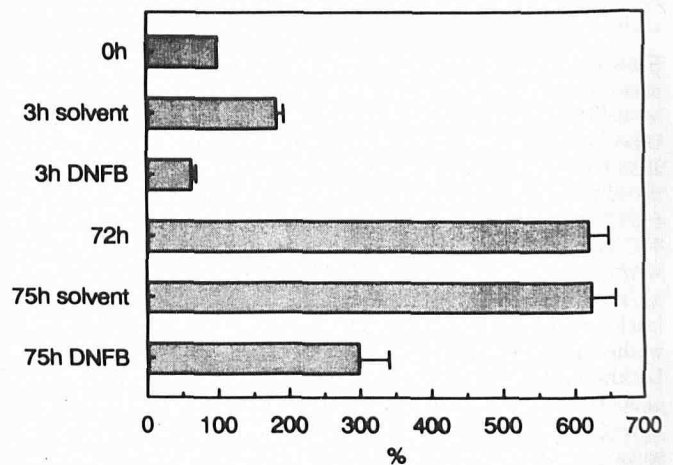

Figure 3. Downregulation of IA molecule expression of freshly prepared and 3-d cultured LC during short-term culture in the presence of DNFB. Freshly prepared EC and EC precultured for 72 h were short-term cultured for 3 h in the presence of 5 μg/ml DNFB or 0.1% acetone as solvent control. The relative IA expression of cultured LC is shown as percent of the IA expression of freshly prepared LC. The mean and SEM of four experiments are shown.
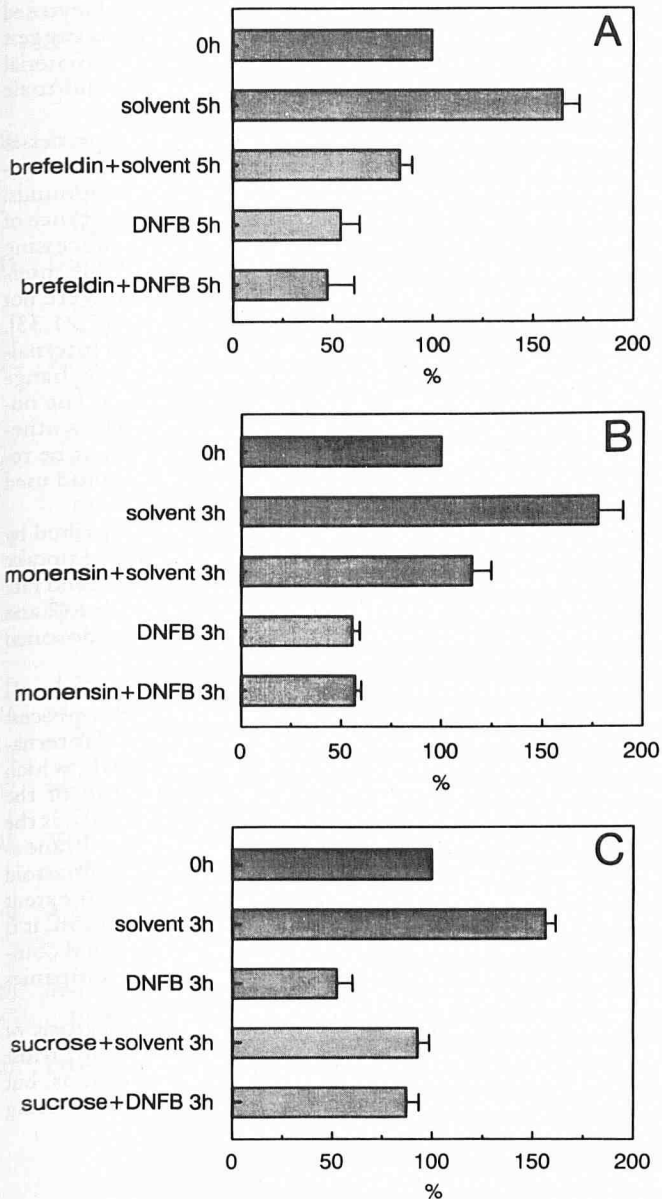
Figure 5. Clearance of antibody-labeled IA molecules on the cell surface of LC in the presence of DNFB. EC precultured for 1 d were labeled with MoAb MK-D6 and cultured in the presence of DNFB *(dotted line)* or solvent *(solid line)*. The remaining amount of cell-surface–expressed antibody was estimated by FCM and expressed as percent of the binding rate at the beginning of the experiment. The mean and SEM of four experiments are shown.

finding indicated that the acid wash was sufficient to remove cell-surface–bound antibody complexes. We therefore conclude that the observed enzyme activity after the short-term culture was indeed associated with endocytosed material. Beside the normally used final concentration of 5 μg/ml DNFB, which resulted in a maximal reduction of Ia expression, lower amounts were tested in this assay and the staining pattern of internalized peroxidase activity was correlated to the viability of LC. This was calculated from parallel experiments carried out with FITC-labeled second-step antibodies instead of peroxidase-labeled antibodies and PI staining of dead cells before subsequent FACScan analysis. Even low concentrations of DNFB (down to 0.5 μg/ml), which had no influence on viability of LC, resulted in a staining pattern represented by Fig 6*B*. In addition, strictly subtoxic doses of DNCB (0.5 μg/ml), TNCB (0.1 μg/ml), and $K_2Cr_2O_7$ (250 μg/ml) caused a similar staining pattern. Subtoxic amounts of SDS (up to 50 μg/ml) resembled untreated or solvent-treated cells (Fig 6*A*), whereas toxic concentrations (100 μg/ml) resulted in a diffuse distribution pattern comparable to Fig 6*B*.

DISCUSSION

In this report we show that sensitizing concentrations of contact allergens [12,23,24] with different chemical structures are able to reduce the expression of MHC class II molecules of LC after in vivo application on murine skin for 3 h. The observed decrease in MHC class II expression could not be explained by an increased cellular death of LC and was detectable independently from toxic effects. Both isotypes of Ia-antigen as well as the CD45-antigen were involved, which may reflect the general exchange of cell surface membranes under the influence of contact allergens as seen in electronmicroscopical studies [12]. SDS was also able to reduce the Ia-antigen expression but only in combination with distinct toxic effect resulting in irreversible cellular damage. Further studies have to elucidate the underlying mechanism of depressed MHC class II molecule expression under these conditions. However, initial downregulation of Ia-antigen under the influence of contact allergens cannot be explained by toxic effect. This assumption is also supported by our in vitro studies with internalized MoAb to MHC class II molecules. In addition, recent data from our laboratory (unpublished observations) suggest profound differences in the subcellular organization of endocytotic compartments of LC under the influence of contact allergens and irritants, respectively.

For in vitro experiments DNFB was used as a model compound to study the influence of contact allergens on the Ia-antigen expression of LC during short-term culture. DNFB-treated fresh LC as well as LC precultured for 3 d in bulk EC preparations showed a reduced Ia-

Figure 4. The downregulation of IA molecule expression under the influence of DNFB is not inhibited by brefeldin A or monensin but blocked under hypertonic conditions. *(A)* EC from Balb/c mice were cultured in the presence of 1 μg/ml/brefeldin A for 2 h followed by a short-term culture with 5 μg/ml DNFB or 0.1% acetone and subsequent FCM analysis. *(B)* A similar experiment was carried out without preculture for 2 h with 10 μM monensin instead of brefeldin A. *(C)* EC were precultured for 15 min in the presence or absence of 0.45 M sucrose and 5 μg/ml DNFB or 0.1% acetone were added for 3 h at 37°C. Mean and SEM of three experiments are shown.

seen in the presence of DNFB. To show that this material is internalized and to obtain first insights into its fate we performed internalization studies with peroxidase-labeled antibodies. LC precultured for 1 d and labeled with anti-IA and peroxidase-coupled second-step antibodies showed an accumulation of internalized peroxidase activity into large granular structures with a faint cytoplasmic staining after short-term culture for 1 h in the presence of solvent or without any treatment (Fig 6*A*). In contrast, DNFB-treated cells exhibited pronounced submembraneous accumulation of peroxidase activity with diffusely stained cytoplasm (Fig 6*B*). Appropriate controls carried out by applying the acid wash procedure immediately after labeling showed no significant staining of LC (not shown). This
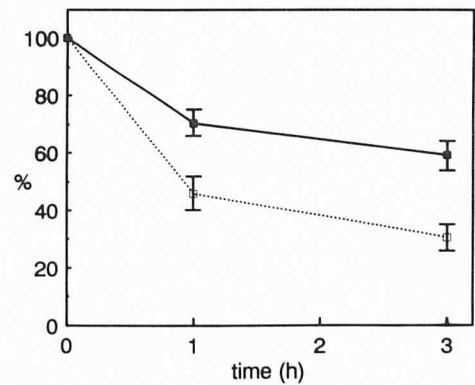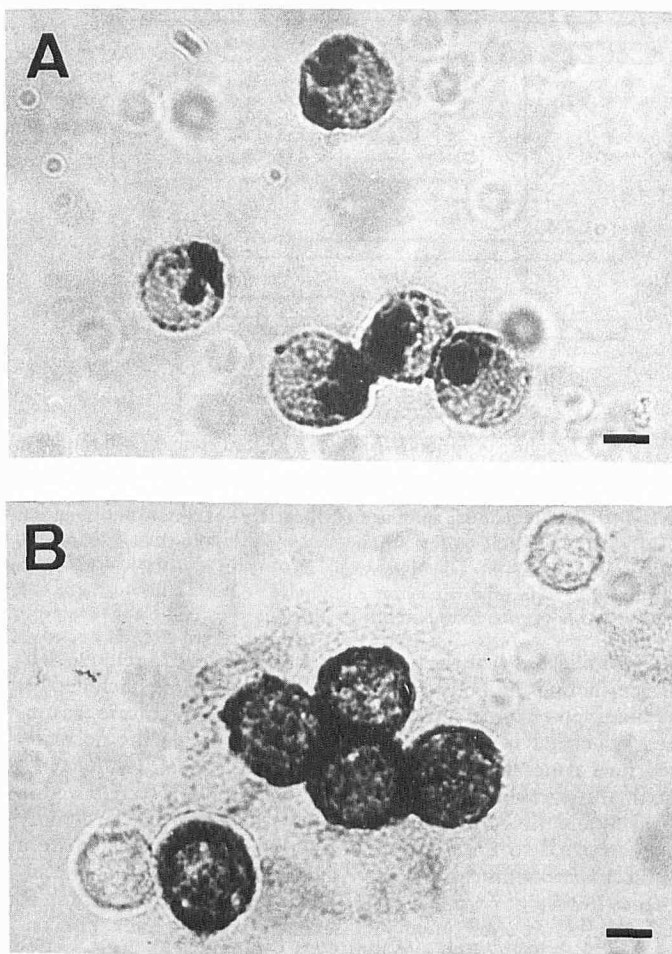
Figure 6. Distribution pattern of internalized peroxidase-labeled antibody-IA molecule complexes of haptenized and solvent-treated LC after short-term culture. LC precultured for 1 d were enriched by panning, labeled with MoAb MK-D6 and peroxidase-coupled second-step antibody, and cultured for 1 h in the presence of 0.1% acetone *(A)* as control or 5 μg/ml DNFB *(B)*. The remaining intracellular peroxidase activity was visualized after an acid wash and is represented by the dark structures. *Bar, 4.2 μm.*

and toxic doses of SDS influenced the distribution of endocytosed antibody-labeled IA-antigen. Our latest unpublished results suggest profound differences in the subcellular organization of this material in the presence of subtoxic amounts of contact allergens and toxic doses of irritants, respectively.

Qualitative and quantitative alterations in endocytotic processes under the influence of contact allergens might have important implications for the processing and presentation of these compounds. Conflicting data exist about the degree as well as the importance of internalization of MHC class II molecules for antigen processing and presentation. Whereas some authors reported remarkable internalization of MHC class II molecules [16,27–32], others were not able to detect sufficient endocytosis of these molecules [17,21,33]. Although evidence was presented for an important role of internalized class II molecules for antigen presentation by peptide exchange in endosomal compartments [28,34], most authors support the notion that processed antigen is mainly associated with newly synthesized class II molecules [19,20,33]. These differences might be related to the type of cells investigated, as well as to the method used to detect internalization.

For human LC RME of HLA-DR antigen has been described by Hanau et al [10] and Girolomoni et al [11], who observed the uptake of antibody-labeled HLA-DR molecules. They found a reduced rate of internalization for cultured LC in comparison to fresh LC and more strikingly a failure of cultured LC to acidify their endosomal compartments.

Our findings suggest that LC are able to internalize MHC class II molecules and show that contact allergens modulate this process profoundly. The underlying mechanism of this augmented internalization in the presence of contact allergens is probably RME, which increases after contact allergen application [12]. The fate of the internalized MHC molecules is unknown. Of special interest is the question of whether these molecules recycle to the cell membrane as reported for an Epstein-Barr-virus–transformed lymphoblastoid cell line [31] and murine B lymphoma cells [32], and to what extent antigen exchange might occur during this process. In addition, it is unknown whether contact allergen gains access to endosomal compartments during this internalization and whether it accompanies the endocytosed class II molecules.

Taken together, our data show that augmented endocytosis of MHC class II molecules is an early event of LC activation. Most likely, this process is not mediated solely by contact allergens, but for these compounds it might have consequences for the assembling of an immunogenic allergen-MHC complex.

and CD45-antigen expression similar to the results of the in vivo experiments.

Our experiments with brefeldin A and monensin revealed that an abolished transport of newly synthesized and recycled class II molecules to the cell membrane is not the cause for the downregulation of Ia expression in the presence of DNFB.

Hypertonic conditions (by addition of 0.45 M sucrose) in our in vitro cultures of haptenized EC prevented the downregulation of Ia molecule expression after treatment with DNFB. This indicates that an active cellular mechanism like RME, which is inhibited under hypertonic conditions by blocking the formation of clathrin-coated pits [22], is underlying our observations and not a passive process like an alteration of binding sites for the MoAb by DNFB or an increased passive proteolytic degradation of MHC class II molecules. Such passive alterations of immunogenic peptide-MHC structures on antigen-presenting cells were detected by functional assays after treatment with $NiSO_4$ [25]. In this context it should be noticed that an increase of class II molecules in endocytotic structures as well as a decrease of cell-membrane–associated Ia-antigen was found for in vivo haptenized LC by electronmicroscopical methods ([26] and G. Kolde, personal communication).

Beside this indirect evidence for augmented endocytosis of class II molecules we were able to show that the clearance of MoAb-labeled cell-surface–expressed IA molecules is accelerated in the presence of DNFB and that subtoxic amounts of different contact allergens

REFERENCES

1. Shimada S, Caughman SW, Sharrow SO, Stephany D, Katz SI: Enhanced antigen-presenting capacity of cultured Langerhans cells is associated with markedly increased expression of Ia antigen. J Immunol 139:2551–2555, 1987

2. Witmer-Pack MD, Valinsky J, Olivier W, Steinmann RM: Quantitation of surface antigens on cultured murine epidermal Langerhans cells: rapid and selective increase in the level of surface MHC products. J Invest Dermatol 90:387–394, 1988

3. Puré E, Inaba K, Crowley MT, et al: Antigen processing by epidermal Langerhans cells correlates with the level of biosynthesis of major histocompatibility complex class II molecules and expression of invariant chain. J Exp Med 172:1459–1469, 1990

4. Kämpgen E, Koch N, Koch F, et al: Class II histocompatibility complex molecules of murine dendritic cells: synthesis, sialylation of invariant chain, and antigen processing capacity are down-regulated upon culture. Proc Natl Acad Sci USA 88:3014–3018, 1991

5. Becker D, Reske-Kunz AB, Knop J, Reske K: Biochemical properties of MHC class II molecules endogenously synthesized and expressed by mouse Langerhans cells. Eur J Immunol 21:1213–1220, 1991

6. Schuler G, Steinman RM: Murine epidermal Langerhans cells mature into potent immunostimulatory dendritic cells in vitro. J Exp Med 161:526–546, 1985

7. Romani N, Lenz A, Glassel H, et al: Cultured human Langerhans cells resemble lymphoid dendritic cells in phenotype and function. J Invest Dermatol 93:600–609, 1989

8. Teunissen MBM, Wormmeester J, Krieg ST, et al: Human epidermal

Langerhans cells undergo profound morphologic and phenotypical changes during in vitro culture. J Invest Dermatol 94:166–173, 1990

9. Aiba S, Katz SI: Phenotypic and functional characteristics of in vivo-activated Langerhans cells. J Immunol 145:2791–2796, 1990

10. Hanau D, Fabre M, Schmitt DA, et al: Human epidermal Langerhans cells cointernalize by receptor-mediated endocytosis "nonclassical" major histocompatibility complex class I molecules (T6 antigens) and class II molecules (HLA-DR antigens). Proc Natl Acad Sci USA 84:2901–2905, 1987

11. Girolomoni G, Cruz PD Jr, Bergstresser PR: Internalization and acidification of surface HLA-DR molecules by epidermal Langerhans cells: a paradigm of antigen processing. J Invest Dermatol 94:753–760, 1990

12. Kolde G, Knop J: Different cellular reaction patterns of epidermal Langerhans cells after application of contact sensitizing, toxic, and tolerogenic compounds. A comparative ultrastructural and morphometric time-course analysis. J Invest Dermatol 89:19–23, 1987

13. Kappler JW, Skidmore B, White J, Marrack P: Antigen-inducible, H-2-restricted, interleukin-2-producing T cell hybridomas. J Exp Med 153:1198–1214, 1981

14. Ozato K, Mayer N, Sachs DH: Hybridoma cell lines secreting monoclonal antibodies to mouse H-2 and Ia antigens. J Immunol 124:533–540, 1980

15. Springer T, Galfrè G, Secher DS, Milstein C: Monoclonal xenogeneic antibodies to murine cell surface antigens: identification of novel leukocyte differentiation antigens. Eur J Immunol 8:539–551, 1978

16. Machy P, Bizozzero J-P, Reggio H, Leserman L: Endocytosis and recycling of MHC-encoded class II molecules by mouse B lymphocytes. J Immunol 145:1350–1355, 1990

17. Mehringer JH, Cullen SE: Internalization of Ia molecules by antigen-presenting B cells is limited. J Immunol 145:2064–2069, 1990

18. Willis CM, Stephens CJM, Wilkinson JD: Differential effects of structurally unrelated chemical irritants on the density and morphology of epidermal CD1+ cells. J Invest Dermatol 95:711–716, 1990

19. Adorini L, Ullrich SJ, Appella E, Fuchs S: Inhibition by brefeldin A of presentation of exogenous protein antigens to MHC class II-restricted T cells. Nature 346:63–66, 1990

20. St-Pierre Y, Watts TH: MHC class II-restricted presentation of native protein antigens by B-cells is inhibitable by cycloheximide and brefeldin A. J Immunol 145:812–818, 1990

21. Davis JE, Cresswell P: Lack of detectable endocytosis of B lymphocyte MHC class II antigens using an antibody-independent technique. J Immunol 144:990–997, 1990

22. Heuser JE, Anderson RGW: Hypertonic media inhibit receptor-mediated endocytosis by blocking clathrin-coated pit formation. J Cell Biol 108:389–400, 1989

23. Fachet J, Andó I: Genetic control of contact sensitivity to oxazolone in inbred, H-2 congenic and intra-H-2 recombinant strains of mice. Eur J Immunol 7:223–226, 1977

24. Mor S, Ben-Efraim S, Leibovici J, Ben David A: Successful contact sensitization to chromat in mice. Int Arch Allergy Appl Immun 85:452–457, 1988

25. Romagnoli P, Labhardt AM, Sinigaglia F: Selective interaction of Ni with an MHC-bound peptide. EMBO 10:1303–1306, 1991

26. Kolde G, Knop J: Epidermal Langerhans cells internalize and re-express surface Ia molecules in allergic contact dermatitis (abstr). Arch Dermatol Res 283:19–20, 1991

27. Harding CV, Unanue ER: Antigen processing and intracellular Ia. Possible roles of endocytosis and protein synthesis in Ia function. J Immunol 142:12–19, 1989

28. Harding CV, Roof RW, Unanue ER: Turnover of Ia-peptide complexes is facilitated in viable antigen-presenting cells: biosynthetic turnover of Ia vs. peptide exchange. Proc Natl Acad Sci USA 86:4230–4234, 1989

29. Neefjes JJ, Stollorz V, Peters PJ, Geuze HJ, Ploegh HL: The biosynthetic pathway of MHC class II but not class I molecules intersects the endocytic route. Cell 61:171–183, 1990

30. Harding CV, Unanue ER, Slot JW, Schwartz AL, Geuze HJ: Functional and ultrastructural evidence for intracellular formation of major histocompatibility complex class II-peptide complexes during antigen processing. Proc Natl Acad Sci USA 87:5553–5557, 1990

31. Reid PA, Watts C: Cycling of cell-surface MHC glycoproteins through primaquin-sensitive intracellular compartments. Nature 346:655–657, 1990

32. Salamero J, Humbert M, Cosson P, Davoust J: Mouse B lymphocyte specific endocytosis and recycling of MHC class II molecules. EMBO 9:3489–3496, 1990

33. Guagliardi LE, Koppelman B, Blum JS, Marks MS, Cresswell P, Brodsky FM: Co-localization of molecules involved in antigen processing and presentation in an early endocytic compartment. Nature 343:133–139, 1990

34. Adorini L, Appella E, Doria G, Cardinaux F, Nagy ZA: Competition for antigen presentation in living cells involves exchange of peptides bound by class II MHC molecules. Nature 342:800–803, 1989

DERMATOLOGY NURSES' ASSOCIATION SETS 1992 SUMMER MEETING

The Dermatology Nurses' Association (DNA) will hold its second annual Summer Meeting June 12–13, 1992, at the Loews New York Hotel, New York City. The program will feature 2 full days of educational sessions and exhibits, including a focus on international nursing and DNA's popular "Dermatology Core Curriculum."

On Friday, June 12, timely updates on nursing in a pluralistic society, dermatology nursing in the United Kingdom, skin and HIV, phototherapy, pediatric AIDS, and surgical intervention in nail care will be presented.

On Saturday, June 13, the Dermatology Core Curriculum will provide a foundation of knowledge for individuals new to the dermatology field as well as update and broaden the knowledge base for those with experience.

Educational exhibits will be open during an evening reception on Friday, and during a continental breakfast and coffee break Saturday morning.

Continuing education credit will be offered for the 2-day program. Nearly 800 dermatology nursing professionals attended DNA's Annual Convention in 1991.

DNA is a 1,700-member specialty nursing organization dedicated to developing and fostering the highest standards of dermatologic nursing care. For DNA Summer Meeting registration information, contact the DNA Executive Secretary, North Woodbury Road/Box 56, Pitman, NJ 08071; (609) 582-1915; FAX (609) 589-7463.