Keratinocyte Expression of MHC Class II Antigens in Allergic Sensitization and Challenge Reactions and in Irritant Contact Dermatitis*

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Keratinocytes expressed major histocompatibility complex class II antigens during the development of irritant contact dermatitis, and during the induction of contact hypersensitivity, as well as in established allergic contact dermatitis. A battery of anti-class II monoclonal antibodies, some of which are specific for class II subregion products (DP, DQ, DR), was used in an immunohistochemical study of the sequential changes in the allergic challenge reactions to dinitrochlorobenzene (DNCB) and nickel, the irritant response to anthralin, and the induction of sensitization to DNCB. The induction of keratinocyte class II expression paralleled the influx of Leu-3a$^+$ T cells into the skin and had occurred by 24 or 48 h in each type of reaction. Differential expression of class II subregion products on keratinocytes was noted: DR was the most frequently expressed molecule, followed by DP and DQ, although in the irritant response, DP expression was not observed. The importance of these observations can be decided only by functional studies. *J Invest Dermatol 88:11–16, 1987*

Major histocompatibility complex (MHC) class II antigens are cell surface glycoproteins concerned with antigen presentation and the elicitation of T lymphocyte-dependent immune responses. The class II antigens are predominantly expressed on immune-competent cells such as B lymphocytes, activated T lymphocytes, macrophages, Langerhans cells, and endothelial cells, and are more restricted in distribution than the ubiquitous cell surface class I antigens.

The genetic organization of the MHC class II antigens is complex. Class II antigens are encoded in a segment of the MHC on chromosome 6, the genes being organized into 3 subregions (loci), now known as DP, DQ, and DR. At the cell surface, each class II molecule exists as a dimer of one α and one β chain, with the DP, DQ, and DR β chain genes and the DQ α chain genes being highly polymorphic [1]. The number of genes and the number of dimers may vary in different haplotypes; in addition there may be more class II genes and gene products than those recognized at present, making the system even more intricate [2].

In the normal epidermis, class II antigens are expressed by Langerhans cells and by some keratinocytes, especially the cells of the acrosyringeal epithelium [3,4]. Keratinocyte expression occurs in certain immunologically mediated conditions including graft-versus-host disease [5], lichen planus [6], and allergic contact dermatitis [7]; and in some neoplastic disorders, e.g., mycosis fungoides [7,8] or B-cell lymphoma [9]. Recently it was shown to occur in other dermatoses, including various infections, lupus erythematosus, and pityriasis lichenoides [10]. In vitro studies have shown that gamma interferon induces DR synthesis in cultured human keratinocytes [11,12], explaining the finding of Ia synthesis by keratinocytes in mouse graft-versus-host disease [13]. The above dermatoses are characterized by an influx of T lymphocytes into the dermis and often the epidermis, and this has led to the suggestion that these cells produce gamma interferon which induces production of class II antigens by keratinocytes [14,15].

There are now available a large number of monoclonal antibodies against different determinants of class II antigens, and some of these are specific either for DR or DP or DQ antigens [16]. This permits the study of keratinocyte expression of products of the different class II genes at a refined level. Most of the reagents used in the present study have been characterized extensively using MHC deletion mutant cell lines [16,17] and by biochemical means [18,19], and this is central to a circumspective analysis of the results.

MATERIALS AND METHODS

Subjects Four groups of subjects were studied. Each of the first group of 12 volunteers (11 males, 1 female; mean age 51 years) was sensitized to 2,4-dinitrochlorobenzene (DNCB) by applying 2 patches impregnated with 100 μg DNCB (prepackaged and kindly provided by Institut Merieux, Lyon, France) to forearm skin for 24 h. Biopsies were taken under 2% lidocaine local anesthesia from the sensitization sites at 1, 6, 24, and 48 h and 3, 8, 10, and 14 days. In the second set, 21 volunteers (18 males, 3

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Abbreviations:

DNCB: 2,4-dinitrochlorobenzene

MHC: major histocompatibility complex

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females; mean age 57 years) who previously had been sensitized to DNCB were challenged on forearm skin with patches impregnated with 40 μg DNCB; biopsy specimens were taken at 1, 3, 6, 12, 24, and 48 h after challenge. The third group, consisting of 16 nickel-sensitive subjects (1 male, 15 female; mean age 45 years) was challenged with 5% nickel sulfate in 60 mg petrolatum (Trolab, Hermal-Chemie Kurt Hermann, Hamburg, West Germany) using a 12-mm Finn chamber on Scanpor (Epitest Ltd Oy, Helsinki, Finland) and biopsies were performed at 1, 4, 6, 8, 24, and 48 h. In the final set of 9 volunteers (6 males, 3 females; mean age 55 years), patch testing with 0.1% anthralin (and 0.5% salicylic acid) in 60 mg petrolatum was effected and biopsies were done at 8 and 48 h. Controls of petrolatum alone, 0.5% salicylic acid in petrolatum, and 5% nickel sulfate in petrolatum (in non-nickel-sensitive subjects), applied under occlusion for 8 h, were employed; 3 biopsies were taken for each of these groups. In each subject, for all the studies outlined, a control biopsy specimen of nonchallenged skin was secured, and no volunteer was biopsied more than 3 times. All of these investigations had been approved by the Physicians' Ethical Committee of our hospital, and each subject gave informed written consent.

Immunoperoxidase Technique Each biopsy specimen was immediately snap-frozen in liquid nitrogen, stored frozen at −70°C, and subsequently embedded in O.T.C. compound (Miles Laboratories Inc, Illinois) prior to use. Cryocut 5 μm-thick sections were placed on poly-L-lysine-coated slides, fixed in acetone at room temperature for 20 min, and incubated with 20% normal rabbit serum in Tris-buffered saline (0.001 M, pH 7.6) for 10 min. They were then either incubated for 1 h at room temperature with the monoclonal antibody diluted in 20% normal rabbit serum in Tris-buffered saline (nickel and anthralin studies) or incubated overnight with similarly prepared monoclonal antibodies but at a greater dilution and at 4°C (DNCB studies) (Table I). After washing with Tris-buffered saline, they were incubated with rabbit antimouse immunoglobulin conjugated with horseradish peroxidase (Dakopatts a/s, Glostrup, Denmark) for 45 min. Development of the enzyme reaction was achieved using 0.03% diaminobenzidine (Sigma Chemical Company Ltd, Poole, England) with 0.068% imidazole (BDH Chemicals Ltd, Poole, England) and 0.02% hydrogen peroxide in Tris-buffered saline. Mayer's hemalum was used as a counterstain.

Monoclonal Antibodies The monoclonal antibodies used, their sources, immunoglobulin subclasses, specificities, and dilutions, are shown in Table I. Not every biopsy specimen was tested against the full anti-class II antigen battery, and in particular the earliest specimens from the DNCB- and nickel-sensitive subjects were tested only with DA6.147, DA6.164, DA6.231, and OKIa1.

DA6.147, DA6.231, and OKIa1 recognize "framework" determinants of class II molecules with specificities as previously described (16,17,19–21). DA6.164 (DR), L 243 (DR), B 7/21 (DP), Leu-10 (DQ), and BT 3/4 (DQw1) are antibodies specific for products of a single locus. Some of the determinants recognized are polymorphic (DA6.164, Leu-10, and BT 3/4) [16,17,19,22,23], but other antibodies recognize class II antigens in all individuals (L 243 and B 7/21).

All sections were also reacted with Leu-2a, Leu-3a, Leu-M3, and Leu-6, thus allowing phenotypic characterization of the cellular infiltrates. In addition, an irrelevant mouse monoclonal antibody DA6.127 (directed against human immunoglobulin M) was used as a control in the DNCB sensitization study and in a few instances in the nickel challenge study. In the remaining experiments, the control consisted of using 20% normal rabbit serum in Tris-buffered saline, in place of the first monoclonal antibody.

Evaluation of Keratinocyte Class II Antigen Expression The DNCB sensitization and challenge sections were examined by M.M.C., and the nickel challenge and anthralin test sections were analyzed by D.J.G. Three distinct staining patterns were identified; all were membranous in type. The most common was a sheetlike ("chickenwire") involvement of keratinocytes in one or several parts of the malpighian layer. Staining at or adjacent to an acrosyringium or a hair follicle was also a distinctly recognizable pattern. The third type of reactivity was found surrounding individual Langerhans cells, in a different pattern and with more widespread distribution than could be explained simply by staining on dendritic processes. Reactivity, when present, could involve a partial to a full thickness of the malpighian layer, and the length of the segment involved could also vary, although complete involvement of all keratinocytes on a section was not seen. When assessing a biopsy specimen, the full length of the epidermis was examined usually on 3 sections. A biopsy was regarded as positive if the keratinocyte staining in a chickenwire

Table I. The Battery of Monoclonal Antibodies

Monoclonal Antibody	Origin	Immunoglobulin Subclass	Determinant Reactivity	Working Dilution	
				1-Hour Incubation	Overnight
DA6.231[a]	K. Guy [20]	IgG_1	DR + DP + DQ	Neat	1/10
OK Ia1[b]	Ortho[c] [17]	IgG_2	DR	1/20	1/100
DA6.164[a,d]	K. Guy [19]	IgG_1	DR β chain	Neat	1/10
DA6.147[a]	K. Guy [20]	IgG_1	DR α chain + DQ	Neat	1/10
L 243[a]	L.A. Lampson, R. Levy	IgG_{2a}	DR β chain	1/20	1/50
B 7/21[a]	I.S. Trowbridge [19,23]	NK	DP	Neat	Neat
Leu-10[e]	Becton-Dickinson[f]	IgG_1	DQ	1/20	1/100
BT 3/4[b]	G. Corte [22]	IgG_1	DQw1	1/5,000	1/48,000
Leu-2a[g]	Becton-Dickinson[f]	IgG_1	Cytotoxic/suppressor T cell	1/40	1/100
Leu-3a[g]	Becton-Dickinson[f]	IgG_1	Helper/inducer T cell	1/40	1/100
Leu-M3[e]	Becton-Dickinson[f]	IgG_{2b}	Monocyte/macrophage	1/20	1/100
Leu-6[a]	Becton-Dickinson[f]	IgG_{2b}	Langerhans cell/common thymocyte	1/20	1/200
DA6.127[a]	K. Guy	IgG_1	Immunoglobulin M	Neat	Neat

[a]Culture supernatant.
[b]Ascites.
[c]Ortho Diagnostic Systems Inc, Raritan, New Jersey.
[d]Not reactive with DR7.
[e]Serum/ascites.
[f]Becton Dickinson Monoclonal Center Inc, Mountain View, California.
[g]Culture supernatant or serum/ascites fluid.
NK = Not known.

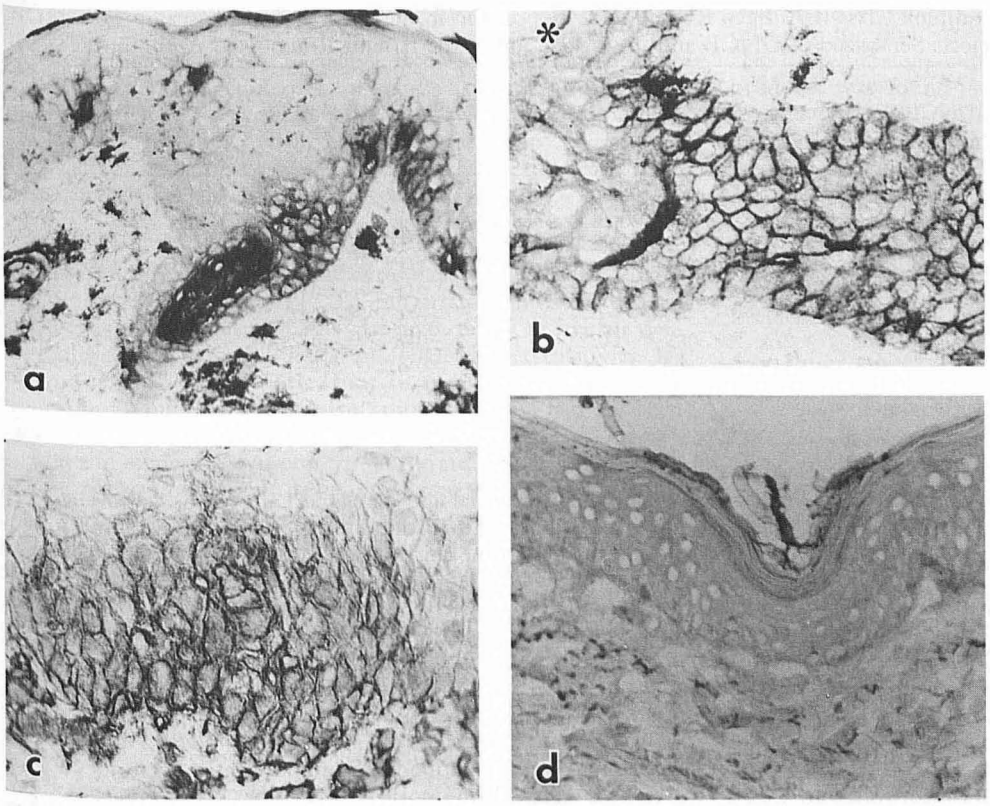

Figure 1. *a,* Periacrosyringeal membranous keratinocyte staining is seen with DA6.164 (DR) in this normal epidermis. Dendritic Langerhans cells are easily distinguished. The acrosyringeal epithelium shows intense reactivity (×320). *b,* Chickenwire-type reactivity with DA6.231 (DP, DQ, DR) is evident on appendageal keratinocytes at 24 h in the induction of sensitization to DNCB. *Asterisk* shows horny layer (×500). *c,* Intense keratinocyte reactivity showing a chickenwire pattern is found with DA6.164 (DR) at 48 h in this nickel allergic reaction (×500). *d,* No keratinocyte reactivity was found at 24 h in the induction of sensitization to DNCB, using the irrelevant control DA6.127, which is of the IgG_1 isotype (×320).

pattern, or less frequently in a periappendageal pattern, was greater than the nonchallenged background. Staining around Langerhans cells alone was not sufficient for a positive result. Segmental involvement of the type normally regarded as significant is shown in Figs 1*b,c* and 2*c,d*.

In the 48-h biopsy specimens from the anthralin irritant reactions of 2 subjects, the reactivity with a battery of monoclonal antibodies of cell bodies within defined areas of epidermis was quantified on serial sections. An eyepiece graticule was used and only positively staining cell bodies were counted (see Table III).

RESULTS

Nonchallenged Skin Some degree of background membranous keratinocyte staining was seen in several nonchallenged biopsy specimens with certain anti-class II antibodies. This was often weak and could easily be overlooked but did not occur with

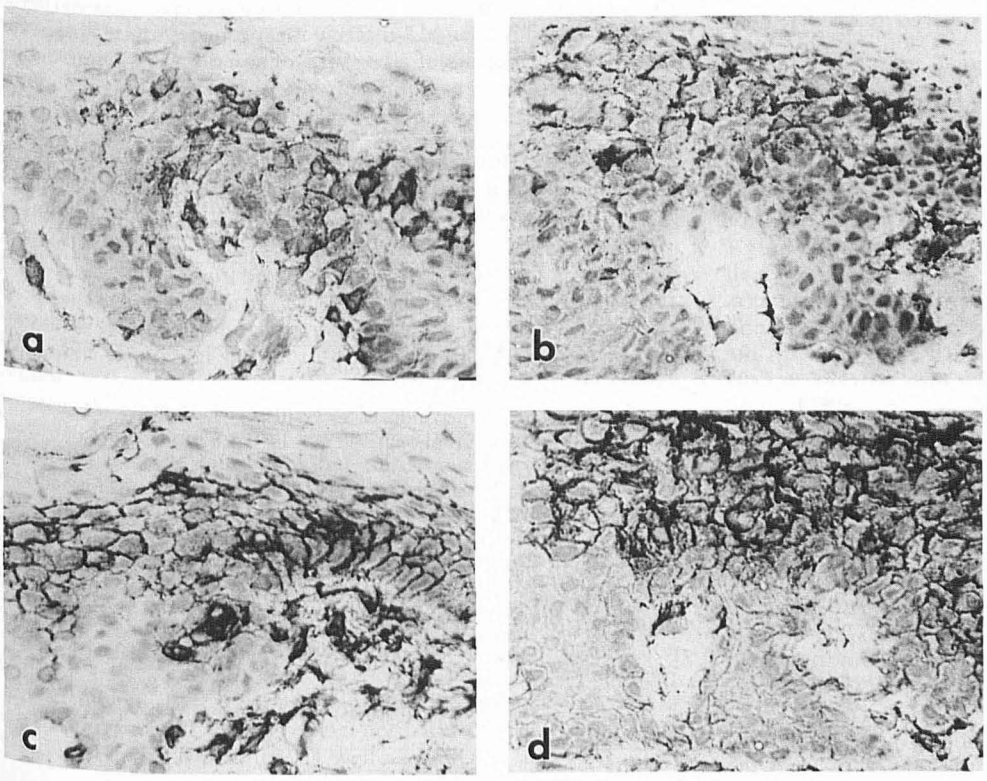

Figure 2. Serial sections through an area of epidermis in the 48-h anthralin irritant reaction. *a,* A moderate number of Leu-3a$^+$ cells are present in the epidermis; some of these may be Langerhans cells (×500). *b,* Moderate reactivity with infiltrating inflammatory cells, but no keratinocyte staining, was noted with OKIa1 (×500). *c,* Chickenwire keratinocyte membrane reactivity with BT 3/4 (DQw1); some of the more heavily staining inflammatory cells are distinguishable in the dermis and epidermis (×500). *d,* More extensive staining, due largely to reactivity on keratinocyte membranes, is evident with DA6.231 (DR, DP, DQ) (×500).

Table II. Major Histocompatibility Complex Class II Antigen Keratinocyte Expression in the Induction of Sensitivity to DNCB, the Challenge Reactions in Subjects Sensitized to DNCB and to Nickel, and in the Irritant Reaction to Anthralin[a]

Monoclonal Antibody	Determinant Reactivity	DNCB Sensitization			DNCB Challenge			Nickel Allergic			Anthralin Irritant	
		<24 h	24 h	72 h	<6 h	6 h	24 h	<24 h	24 h	48 h	8 h	48 h
DA6.231	DP + DQ + DR	0/8	0/4	3/3	1/5	0/3	2/3	1/17	3/5	5/5	1/5	2/3
OKIa1	DR	0/8	2/4	3/3	1/8	3/4	4/4	0/17	0/2	1/3	0/3	0/2
DA6.147	DR (α chain) + DQ	1/7	3/4	3/3	1/5	3/3	3/3	0/15	0/6	0/5	0/4	0/4
DA6.164	DR (β chain)	0/8	1/4	2/3	1/5	2/3	2/3	1/17	1/4	4/4	0/4	3/3
L 243	DR (β chain)	0/5	2/2	1/1	—	—	—	0/4	0/1	2/2	0/3	2/3
B 7/21	DP	0/6	0/3	1/1	—	—	—	0/4	0/2	2/2	0/3	0/3
Leu-10	DQ	0/8	0/4	2/3	0/2	0/2	1/2	0/7	0/2	2/3	0/4	1/4
BT 3/4	DQw1	0/5	0/2	1/1	—	—	—	0/3	—	0/1	0/3	1/3

[a]Expressed as number of positives over total number of subjects examined.

the irrelevant control monoclonal antibody DA6.127, which is of the same isotype as many anti-class II antibodies. The keratinocyte reactivity was most common with DA6.231 (DP, DQ, DR), and DA6.164 (DR), but was less frequent with the other antibodies. The staining was often perifollicular or periacrosyringeal, and the acrosyringeal epithelium was uniformly positive with all anti-class II antibodies (Fig 1*a*). Control biopsy specimens from skin challenged with petrolatum, 0.5% salicylic acid in petrolatum or 5% nickel sulfate in petrolatum (the latter in nonsensitized subjects), gave similar results to the above. We believe that this minor keratinocyte staining in nonchallenged skin is specific [4,24] but it was always less intense, and not as widespread as that seen with subsequent reactions.

Induction of Sensitivity to DNCB At 24 h, expression of class II antigens on keratinocytes was evident with DA6.147, L 243, and OKIa1 (Table II). By 72 h keratinocyte reactivity with antibodies to DP and DQ had occurred, and sections were clearly stained with DA6.231 (Fig 1*b*). The biopsy specimens examined between days 3–14 exhibited reactivity patterns similar to those at 72 h. No reactivity was found with the irrelevant control antibody DA6.127 (Fig 1*d*).

2,4-Dinitrochlorobenzene Challenge Reaction At 6 h after challenge with DNCB, some subjects showed reactivity with certain anti-class II antibodies (Table II). By 24 h, staining with DA6.231 was found, and Leu-10 (DQ) was reactive in 1 of 2 subjects. The 48-h pattern was similar to that seen at 24 h.

Nickel Allergic Challenge Reaction No increase above the background staining was found before 24 h in this group except for 1 subject who reacted with DA6.164 (DR) at 8 h, and another who reacted with DA6.231 also at 8 h (Table II). By 24 h, 3 of 5 subjects demonstrated staining with DA6.231, although only 1 of 4 tested also showed this with DA6.164; the reactions with all other antibodies were unchanged. However, at 48 h all 5 subjects exhibited staining with DA6.231; this was reflected in staining with DR antibodies DA6.164 (Fig 1*c*) and L 243, and with the DP antibody (B 7/21) in all individuals. DQ reactivity with Leu-10 was noted in 2 of 3 biopsy specimens tested but BT 3/4 was negative in the 1 subject assessed. DA6.147 was consistently negative for keratinocyte reactivity in all 5 of the 48-h biopsy specimens processed.

Irritant Dermatitis Induced by Anthralin At 8 h, no change was found except in 1 biopsy specimen which reacted with DA6.231 (DR, DP, and DQ) (Table II). However, by 48 h almost all subjects demonstrated keratinocyte staining with DA6.164 and L 243, and one-third were positive with Leu-10 and BT 3/4. Reactivity at 48 h was noted with DA6.231 in 2 of 3, but none had staining above the background with DA6.147, B 7/21, or OKIa1 antibodies (Fig 2).

Interpretation at 48 h was often made difficult by the infiltration of lymphocytes and macrophages into the epidermis, and the presence of abnormal and damaged Langerhans cells. These cell types may stain with antibodies to class II antigens but keratinocyte staining of the chickenwire pattern was discernible in places because of the geometric angles at the sides of keratinocytes. This was usually closely related to the lymphocytic infiltrate which involved the upper dermis as well as the epidermis. In view of the possibility that the apparent keratinocyte staining was in fact on adherent masses of lymphocytes, Langerhans cells, or macrophages, counts of reactive cell bodies were performed in two 48-h biopsies. The results for subject 1 (Table III; Fig 2) indicate that for anti-class II antibodies thought to show keratinocyte reactivity (as well as being positive with other cells), e.g., DA6.231, DA6.164, or BT 3/4, the counts were higher than the sum of the lymphocyte, macrophage (Leu-M3), and Langerhans cell numbers together. Subject 2 showed similar results with DA6.231 and positive, but less emphatic, changes with DA6.164 and L 243. These findings affirm that enhanced keratinocyte staining was present.

Phenotypic Characterization of the Cellular Infiltrate A lymphocytic infiltrate into the dermis and epidermis was evident at 6–8 h for the DNCB challenge, nickel challenge, and anthralin irritant responses, with Leu-3a-reactive cells predominating over those positive with Leu-2a. These infiltrates increased over 24–48 h, with, by 48 h, Leu-M3-reactive macrophages appearing in the dermis and epidermis of all 3 types of reaction. 2,4-Dinitrochlo-

Table III. Positively Reacting Cell Bodies, Counted per Graticule Area, in One Region of Epidermis, in Serial Sections of Subjects at 48 Hours After the Application of Anthralin to Forearm Skin

Monoclonal Antibody	Determinant Reactivity	Count	
		Subject 1	Subject 2
Leu-2a	Suppressor/cytotoxic T lymphocyte	9	5
Leu-3a	Helper/inducer T lymphocyte	57	34
Leu-M3	Monocyte/ macrophage	0	5
Leu-6	Langerhans cell/ common thymocyte	18	10
DA6.231	DR + DP + DQ	166	64
OKIa1	DR	45	ND
DA6.147	DR (α chain) + DQ	52	23
DA6.164	DR (β chain)	104	50
L 243	DR (β chain)	83	49
B 7/21	DP	55	31
Leu-10	DQ	68	24
BT 3/4	DQw1	94	36

ND, not done.

robenzene sensitization showed a different time sequence: Leu-3a-reactive and, to a lesser extent, Leu-2a-reactive T cells were present in the dermis (in a periappendageal distribution) at 24 h, but an influx into the epidermis, of Leu-3a$^+$ cells was not evident until 72 h. Leu-M3-reactive macrophages were not noted in this reaction until 10 days, when the sensitization flare occurred. In all cases, some of the Leu-3a$^+$ cells in the epidermis may have been Langerhans cells, rather than lymphocytes [25].

DISCUSSION

We have confirmed that keratinocytes express class II molecules in allergic contact dermatitis [7,26], but in addition we have made the novel observations that this also may occur in irritant dermatitis and during the induction of contact hypersensitivity. Irritant dermatitis was reported not to produce keratinocyte Ia expression in mice [26], but sensitization induction had not been studied previously. Our findings of a predominantly Leu-3a$^+$ dermal and epidermal lymphocytic infiltrate, beginning at 6–8 h for the allergic and irritant reactions, agree with those of others who have studied one or both types of response [27,28]. The keratinocyte class II expression largely paralleled the influx of these cells, beginning at 6 h for DNCB challenge, 24 h for DNCB sensitization, and 24–48 h for the nickel-sensitive and anthralin irritant reactions. The onset of DR expression at 24–48 h is similar to that found to be induced by the addition of gamma interferon to normal cultured human keratinocytes [12]. A relationship between keratinocyte DR expression and a dermal infiltrate has also been reported in the tuberculin reaction [29]. Indeed, the suggestion that production of gamma interferon by lymphocytes may induce this class II antigen expression [14,15] is supported by the in vitro finding that gamma interferon enhances DR expression on Langerhans cells [30] and in cultured keratinocytes [11,12]. In vitro exposure of cultured keratinocytes to gamma interferon increases the number of IgG_1 Fc receptors [12], and therefore nonspecific binding of IgG_1 isotype antibodies to keratinocytes is a possibility in the reactions that we have studied. However, keratinocyte expression of L 243 (IgG_{2a}) was observed, and keratinocyte reactivity with IgG_1 isotype antibodies such as DA6.127, Leu-2a, and Leu-3a was not found, making it unlikely that an increase in keratinocyte IgG_1 Fc receptor numbers produced any nonspecific reactivity in these experiments.

Differential expression of the class II subregion products was observed in all reactions. DR was the most frequently expressed and DQ was the least often observed, although in the irritant reaction, DP expression was not found. A similar noncoordinate expression of class II molecules, with a predominance of DR and lesser expression of DQ, has been reported in normal peripheral blood monocytes [31], in B-cell chronic lymphocytic leukemia [32] and, significantly, in cultured keratinocytes after exposure to gamma interferon [12]. DQ expression, in addition to DR, is necessary for macrophages to present antigen to T cells [31], and DQ$^+$ macrophages are better stimulators in an autologous mixed lymphocyte reaction than DQ$^-$ macrophages [31]. Thus the various class II subregion gene products may have different biologic roles. No functional significance has been demonstrated for keratinocytes expressing class II molecules: in the only functional study reported, Ia$^+$ keratinocytes from mice with graft-versus-host disease did not exhibit any antigen-presenting capacity, although there is a possibility that this was abrogated by the x-irradiation that was given [33]. However, keratinocyte Ia expression was found to correlate with the intensity and duration of murine contact hypersensitivity [34], producing the suggestion that Ia$^+$ keratinocytes may facilitate the movement of lymphoid cells into the skin, in response to antigenic stimulation [34]. Others, though, have shown that in vitro DR expression by malignant melanoma cells can inhibit autologous lymphocyte stimulation [35].

Keratinocyte class II antigen expression may thus occur during the development of irritant contact dermatitis and in the induction of contact hypersensitivity, as well as with the allergic contact challenge reaction. Its onset paralleled the influx of Leu-3a$^+$ T cells into the skin, suggesting that lymphocytes may induce the class II expression. Differential expression of class II subregion products was found, with DR being the most frequently expressed molecule. Further studies are needed to show whether keratinocyte expression of class II antigens is of functional significance.

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