Acta Derm Venereol (Stockh) 1991; 71: 377–383

Immunocytochemical Analysis of Early Focal Cellular Infiltrates in Experimental Oral Contact Hypersensitivity

GUNNAR WARFVINGE and ÅKE LARSSON

Department of Oral Pathology, School of Dentistry, Lund University, Malmö, Sweden

The cellular infiltrates of early contact hypersensitivity reactions to 2,4-dinitrofluorobenzene (DNFB) in rat oral mucosa were phenotypically characterized using serial frozen tissue sections and monoclonal antibodies. $CD2^+CD45RB^-$ lymphocytes and $ED1^+RT1B/D^+$ monocytes/macrophages appeared in focal collections at the epithelium-connective tissue interface 2–6 h after challenge with DNFB. These foci also contained $CD4^+$, $CD5^+$, $CD8^+$, $TCR\alpha\beta^+$ cells. $CD45RB^+$ "naive" T cells were difficult to detect at 2–6 h but appeared in significant numbers at 24 h post-challenge. At this stage, the number of all the other phenotypes also was increased. $CD2^+$ cells were approximately twice as many as $CD5^+$ or $TCR\alpha\beta^+$ respectively, indicating that $TCR\gamma\delta^+$ lymphocytes might be involved. An additional observation was the presence of increased numbers of $CD2^+$ lymphocytes in the oral mucosa of sensitized but not challenged animals. Our findings indicate that the oral mucosa of skin DNFB pre-sensitized animals may be "contact hypersensitivity conditioned" by migration-prone memory T cells, and that these cells may rapidly interact with locally resident epithelial Langerhans' cells following antigen restimulation, creating the very initial antigen-specific part of experimental oral contact hypersensitivity. ***Key words: Oral mucosa; DNFB; Monoclonal antibodies; Mononuclear cells; RT1B/D.***

(Accepted April 23, 1991.)

Acta Derm Venereol (Stockh) 1991; 71: 377–383.

G. Warfvinge, Department of Oral Pathology, School of Dentistry, S-214 21 Malmö, Sweden.

Contact hypersensitivity (CH) is described as the responses of epicutaneous or epimucosal hapten elicitation in a presensitized individual but in spite of recent years' great progress in understanding the molecular biology of lymphocyte activation, the *in vivo* mechanisms governing the induction of CH are poorly understood. The very initial events occurring within the challenged tissue in an antigen-primed individual, involve antigen- or hapten-specific T cell stimulation at the local site of the challenge. Such T cells may belong to a subset of lymphocytes with propensity to migrate or "home" into the tissue from the blood (1), although all of them may not necessarily be antigen-specific.

Normal human epidermis is inhabited by small numbers of T cells of which the majority seem to express CD8 (2). Recent evidence suggests that many of these cells express the αβ heterodimer of the T cell antigen-receptor (TCRαβ) (3) but some of these cells may belong to a minor population of T cells expressing TCRγδ (4, 5). Due to the finding of TCRγδ-bearing intraepithelial lymphocytes within the skin and the intestine, they have been proposed to mediate immunological surveillance of surface epithelia by means of recognizing local membrane alterations (4) but little is known about the possible role of TCRγδ-bearing cells in oral mucosa or in CH.

In previous studies of experimental oral CH (6), we found that MHC class II antigen-expressing cells comprise a sizable proportion of the cellular infiltrates. However, these cells were not further characterized and no studies have followed the temporal development of the elicited reaction in experimental oral CH. The purpose of the present study was therefore to further examine, with monoclonal antibodies (mAbs), the composition and distribution of the pioneering cellular infiltrates within defined focal areas of early, experimentally induced oral CH lesions at various time intervals following challenge of the oral mucosa.

MATERIALS AND METHODS

Animals

Outbred male Sprague-Dawley rats ($RT1^l$ and $RT1^u$) were obtained from Møllegaard Breeding Center, Skensved, Denmark and ALAB, Stockholm, Sweden. The animals weighed 250–350 g and were fed a standard diet with tap water *ad libitum*.

Neurolept analgesia

Etorphine-acepromazine (Immobilon®), 12.5 μg/kg body weight, antidot diprenorphine (Revivon®), 45 μg/kg body weight (7, 8).

Table I. *Murine anti-rat monoclonal antibodies employed for immunohistological analysis*

mAb[a]	Specificity	
ED1		(Monocytes/macrophages, veiled cells)
ED2[b]		(Macrophages)
OX34	CD2	(T cells, NK cells)
W3/25	CD4	(T helper cells, macrophages)
OX19	CD5	(T cells)
OX8	CD8	(T cytotoxic/suppressor cells)
OX22	CD45RB	(190–220 kD, "naive" T cells, B cells)
OX33[b]	CD45	(240 kD, B cells)
R73	TCRαβ	(T cell antigen-receptor αβ)
OX6	RT1B	(homologous to human HLA-DQ)
OX17	RT1D	(homologous to human HLA-DR)

[a] All mAbs were purchased from Serotec, Oxon, England except R73 which was a kind gift from Dr T Hünig, Munich, Germany.

[b] mAbs not included in the consecutive section focus analysis.

Sensitization and tissue preparation

Sensitization to 2,4-dinitrofluorobenzene (DNFB) was performed essentially as described previously (7). In short, 50–100 μl of 0.5 % DNFB was applied to the shaved neck skin, twice at different sites with a one-day interval. Five days later, the buccal mucosa was challenged by the application of 25 μl 0.02% DNFB under neurolept analgesia. Animals were sacrificed in groups of 4, and at 2, 4, 6 or 24 h after challenge, and the buccal mucosae were excised, embedded in OCT compound (Miles Inc, Elkhart, IN, USA) and snap frozen with isopentane at −80°C. Additional buccal mucosa specimens were obtained from animals sensitized to DNFB but not challenged and from non-sensitized animals challenged with DNFB. Buccal mucosa and axillary lymph nodes from non-sensitized, non-challenged animals served as controls.

Immunocytochemistry

Six μm consecutive frozen sections were air-dried, fixed for 10 min in acetone and washed in phosphate buffered saline (PBS) for 20 min. The sections were treated with 0.5% H_2O_2 in methanol for 30 min to block endogenous peroxidase activity followed by 1% normal rabbit serum (Dakopatts, Copenhagen, Denmark) in PBS for 30 min. Primary mAbs (Table I) in PBS were applied for 60 min at room temperature, using optimal dilutions as determined by check-board titration. Following a brief rinse in PBS, the sections were incubated with peroxidase-conjugated rabbit anti-mouse IgG (Dakopatts) in PBS containing 25% normal rat serum (Dakopatts). Peroxidase activity was detected with 0.05% 3,3'-diaminobenzidine (DAB) supplemented with 0.01% H_2O_2 (30 min). The sections were lightly counterstained with Mayer's hematoxylin and mounted.

As it has been indicated that the specific immunostaining may be hampered by H_2O_2/methanol pretreatment (9), some sections were instead pretreated with 0.3% H_2O_2 in PBS for 30 min.

Focus analysis

Three series of consecutive 6 μm frozen sections were taken from each specimen and placed on glass slides so that sections 1, 10 and 19 were on slide #1, sections 2, 11 and 20 on slide #2 etc.. Each slide was then incubated with mAb (Table I), according to the optimal protocol (see above), where each mAb was used on one slide only. Within the first 24 h after DNFB challenge of oral mucosa, inflammatory cells tend to be unevenly distributed in clusters or foci (6). With the aim of analyzing the cellular composition of these foci we utilized serial sectioning. Thus, the focus analysis refers to the study of 9 consecutive sections where the technical quality of the sections permitted the study of a single focus through all of the sections. The cells were counted within rectangles comprising 0.22 μm of epithelium and 0.22 × 0.15 μm of the underlying connective tissue (ct). Two different foci from each of the four different specimens were analyzed, and the mean value from each pair of foci was calculated. The differences between the number of cells stained with the different mAbs were assessed with the Student's t test.

RESULTS

General observations

Sections treated with 0.3% H_2O_2 in PBS showed a more intense specific immunostaining than those pretreated with 0.5% H_2O_2 in methanol, with any given mAb dilution. However, using optimal mAb dilutions as determined by titration, we were unable to detect any difference in the number of cells recorded by the two methods.

Normal oral mucosa

A few isolated $CD2^+$ cells were present in the ct and occasionally, a labelled cell could also be detected basally in the epithelium. Similarly, $RT1B^+$ and $RT1D^+$ cells, with a dendritic appearance, were scattered in the ct but only rarely in the epithelium. Scattered $ED1^+$ cells were seen, only in the ct. Their size was rather small, with a slightly dendritic shape. $ED2^+$ cells which appeared in significant numbers throughout the ct, were larger and more prominently dendritic than the $ED1^+$ cells. $CD4^+$ cells could also be seen, with an appearance and distribution very much like $RT1B^+$ and $RT1D^+$ cells. We were unable to detect more than occasional $CD5^+$ and $CD8^+$ cells, the latter appearing within blood vessels or in adjacent skin areas. We found 2–3 $TCR\alpha\beta^+$ cells basally in the approximately 2 mm long distance of epithelium covering the oral mucous membrane. A few more cells were seen in the ct and

clearly more TCRαβ+ cells appeared in adjacent skin areas, associated with hair bulbs and sebaceous glands. OX22 (CD45RB) mAb stained vessel walls and the basement membrane but no lymphocytic or dendritic cells were labelled.

Non-challenged mucosa of sensitized animals

The oral mucosa of rats, skin sensitized to DNFB but not challenged, largely resembled normal oral mucosa. However, in contrast to normal mucosa, some CD2+, CD4+ and CD8+ as well as a few TCRαβ+ cells were easily detected in the ct (Fig. 1), indicating some degree of immigration of such cells into the tissue. The RT1B/D reactivity did not differ from normal controls.

Challenged mucosa of non-sensitized animals

At 6 h post-challenge, an increased number of CD2+ cells was present in the ct as well as scattered in the epithelium. CD4+ cells were also seen but only occasional CD8+ and TCRαβ+ cells and no evident CD45RB+ cells. RT1B+ and RT1D+ dendritic cells were evenly distributed throughout the ct with a tendency to accumulate parallel to the basement membrane, and isolated cells were also seen basally in the epithelium. By 24 h after challenge, the inflammatory reaction had subsided, with only a few RT1B/D+ cells at the epithelium-ct border and some scattered CD2+ cells in the ct.

Challenged mucosa of sensitized animals

The intensity of the reactions at 2 and 4 h after DNFB challenge showed interindividual variation, ranging from hardly discernible to rather dense infiltrates, with a clear tendency of forming focal collections at the epithelial-ct interface. As a way to quantify these cells, we analyzed series of consecutive frozen sections, covering 50–60 μm in depth. Foci of this magnitude cannot be adequately quantified and since a false impression of the cellular composition of initial reactions could be obtained by exclusively examining more developed, "older" foci, only 6 and 24 h reactions were quantified.

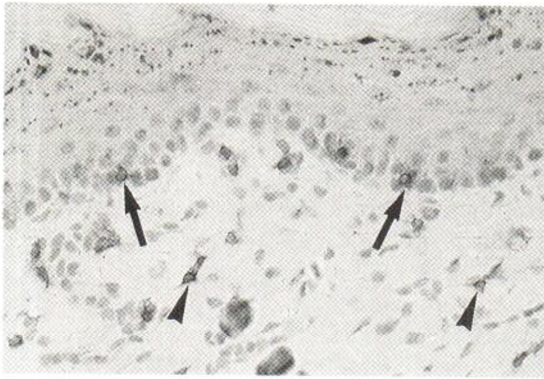
2 and 4 h: In mild reactions, scattered CD2+ cells were seen in the ct and accumulating in small clusters at the epithelium-ct border. These focal clusters were accompanied by RT1B+ and RT1D+ cells but keratinocyte staining could not be convincingly demonstrated. Within the ct, the RT1B/D+ cells often had a dendritic appearance and were situated close to and parallel to the basement membrane. RT1B+ but not RT1D+ cells were seen in close proximity to vessels.

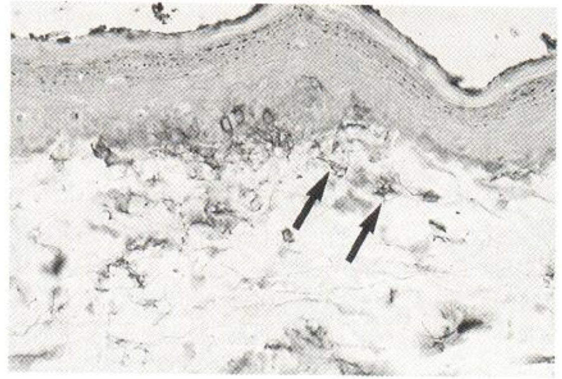
CD4+ cells appeared in numbers almost comparable to CD2+ cells, in the epithelium as well as in the ct (Fig. 2). Some of these CD4+ cells were clearly dendritic. CD5+, TCRαβ+ and occasional CD8+ cells were found scattered as well as within clusters. No CD45RB+ or CD45 (240kD)+ cells were detected.

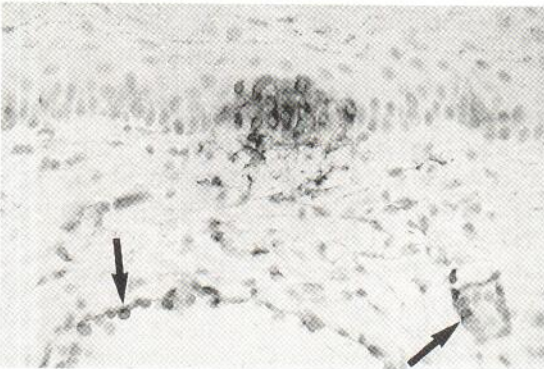
ED1+ cells were not prominent in mild reactions but a major constituent of more developed reactions. Sometimes, ED1+ cells also appeared close to the basement membrane like RT1B/D+ cells. A slight accumulation of ED2+ cells was noted in association with dense infiltrates of lymphocytes.

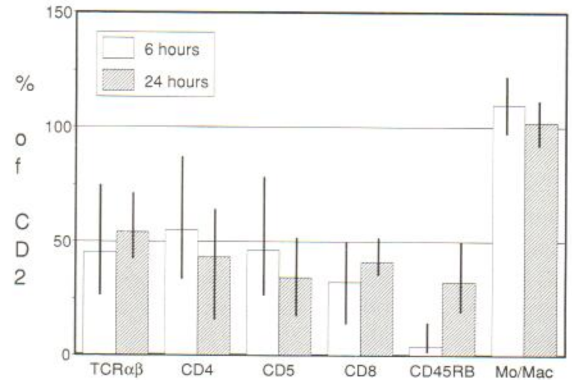
6 h: The inflammatory reactions at 6 h after challenge showed a consistent pattern in all animals, with focal accumulations of cells at the epithelium-ct interface (Fig. 3). The mononuclear cell infiltrates in the ct and within foci were composed of CD2+ and ED1+ cells (Fig. 3). CD2+ cells outnumbered both CD4+, CD5+, CD8+, CD45RB+ and TCRαβ+ cells but not ED1+cells ($p<0.05$). The ratios are given in Fig. 4. ED1+ cells were seen in the epithelium as well as in the ct, where they often formed a band along the basement membrane. The distribution of RT1B+ and RT1D+ cells was very similar to that of ED1+ cells. Focally, delicate ED2+ dendritic structures appeared basally in the epithelium, indicating involvement by ED2+ cells at the site of cell infiltration. No CD45 (240kD)+ cells or PMN were found.

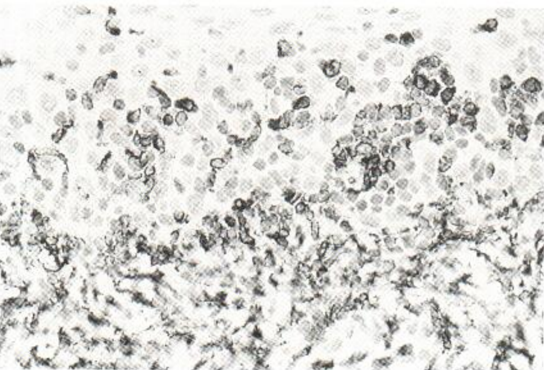
24 h: Reactive cells at 24 h after challenge were seen as dense infiltrates of lymphocytes and non-lymphocytes throughout the juxtaepithelial ct and within the epithelium. Intraepithelial cells sometimes appeared in microvesicles but there was no evidence of ulceration in any of the specimens. Only occasional PMN were detected with hematoxylin-eosin staining. Like the 6 h reactions, the 24 h reactions were dominated by CD2+ together with ED1+ cells (Fig. 5). They appeared in comparable numbers although the ED1+ as well as ED2+ cells were difficult to count in cell-rich areas. Slightly increased numbers of ED2+ cells were found in dense cellular infiltrates as compared to the distribution in the surrounding ct. ED2+ cells also appeared within the epithelium in areas of extensive lymphocyte infiltration (Fig. 6). The proportions of cells which stained with the different mAbs were essentially the same as those at 6 h with CD2+ cells outnumbering all other T cell related phenotypes ($p<0.05$, Figs. 4, 7). The exception was anti-CD45RB, which stained











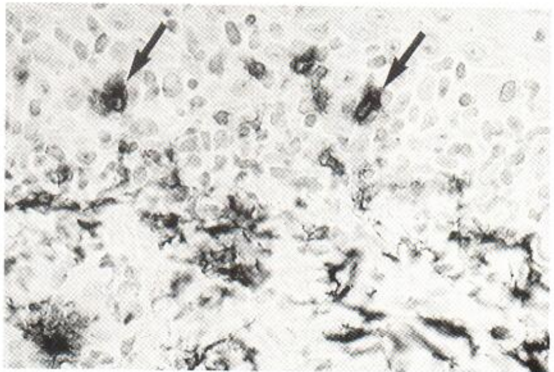
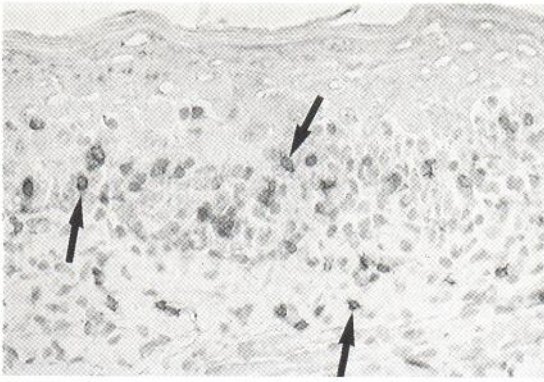
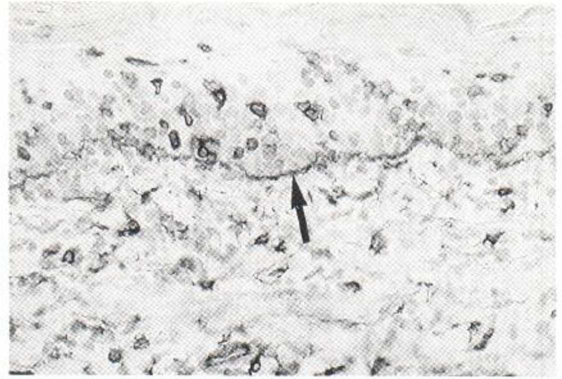


Fig. 1. Frozen section of non-treated oral mucosa from DNFB skin-sensitized animal incubated with OX34 (CD2). $CD2^+$ cells are scattered throughout the ct (*arrowheads*). Reactive cells also appear basally in the epithelium (*arrows*). × 280
Fig. 2. Sensitized animal, oral mucosa 2 h after challenge with DNFB. Section incubated with W3/25 (CD4). Focal collection of $CD4^+$ cells at the epithelium-ct interface. Note the dendritic appearence of some of the cells (*arrows*). × 300
Fig. 3. Sensitized animal, oral mucosa 6 h after DNFB challenge. Section incubated with OX34 (CD2), showing a mononuclear cell focus dominated by $CD2^+$ cells. Note cells migrating through vessel walls (*arrows*). × 280
Fig. 4. Number of cells with different phenotypes expressed as percentage of $CD2^+$ cells. Open/hatched bars represent mean values, and lines the range of individual mean values (n=4).
Fig. 5. Sensitized animal, oral mucosa 24 h after DNFB challenge. Section incubated with ED1, showing a dense mononuclear cell infiltrate basally in the epithelium and in adjacent ct. Reactive cells have a roundish or slightly dendritic appearence. × 300
Fig. 6. Sensitized animal, oral mucosa 24 h after DNFB challenge. Section incubated with ED2. Large dendritic cells are evenly distributed in the juxtaepithelial ct but are also found within the epithelium (*arrows*) in an area of extensive mononuclear cell infiltration. × 400





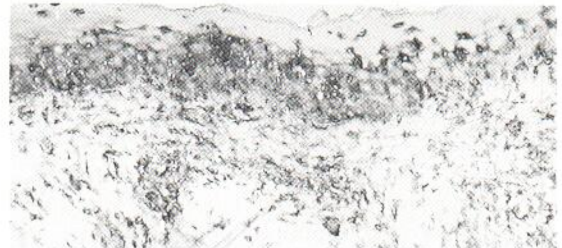


Fig. 7. Sensitized animal, oral mucosa 24 h after DNFB challenge. Section incubated with R73 (TCRαβ). Some reactive cells (*arrows*) appear in an area of extensive mononuclear cell infiltration. × 280

Fig. 8. Sensitized animal, oral mucosa 24 h after DNFB challenge. Section incubated with OX22 (CD45RB). Mononuclear cell infiltrate including some $CD45RB^+$ cells appear in the epithelium and the juxtaepithelial ct. Basement membrane is also stained (*arrow*). × 280

Fig. 9. Sensitized animal, oral mucosa 24 h after DNFB challenge. Section incubated with OX17 (RT1D). $RT1D^+$ cells with ill-defined outlines appear throughout the juxtaepithelial ct. RT1D reactivity in the epithelium is confined to mononuclear and dendritic cells with no convincing staining of keratinocytes. × 140

Fig. 10. Sensitized animal, oral mucosa 24 h after DNFB challenge. Section incubated with OX6 (RT1B). Many reactive cells appear in the epithelium and the juxtaepithelial ct. Keratinocytic RT1B reactivity is also evident basally in the epithelium. × 140

20–40% relative to anti-CD2 as compared to only occasional cells at 6 hr ($p<0.05$, Fig. 8). The $CD45RB^+$ cells were roundishly lymphocyte-like with no more than occasional dendritic cells. Only very few, scattered CD45 $(240kD)^+$ cells were detected.

The number of dendritic $RT1B^+$ and $RT1D^+$ cells was high in the juxtaepithelial ct. Round as well as dendritic cells were seen but individual cells were difficult to count, due to ill-defined outlines. Reactivity to both mAbs was also seen in the epithelium. Here, $RT1D^+$ mononuclear cells appeared isolated or in small clusters, but with no convincing staining of the keratinocytes (Fig. 9). In contrast, keratinocytic staining was clearly found with anti-RT1B, appearing as a diffuse reaction of the cells in areas of $RT1B^+$ mononuclear cell infiltrates (Fig. 10).

DISCUSSION

A small number of T lymphocytes seem to be normal inhabitants of human oral mucosa. They appear basally in the epithelium and in the adjacent (10). In the present study on normal rat oral mucosa, we found small numbers of $CD4^+$, $CD5^+$, $CD8^+$ and $TCR\alpha\beta^+$ cells but no $CD45RB^+$ cells. This is similar to previous findings in human skin (2, 3), indicating the presence of memory type T cells (see below).

The initial CH reaction was identified as minute clusters of $CD2^+$ cells at the epithelium-ct interface by 2 hr after DNFB-challenge. However, increased numbers of $CD2^+$ cells were also present in the oral mucosa of sensitized animals prior to oral DNFB-challenge. As the lesions evolved, there was a continuous infiltration of T cells and monocytes/macrophages but no B cells or PMN. $CD4^+$ cells were predominant in the early oral lesions but $CD8^+$ cells

could always be detected in small numbers. Gawkrodger et al. (11) found a significant increase of CD4$^+$ and CD8$^+$ cells at 4 h following patch-testing of allergic human skin, but in such infiltrates, CD8$^+$ cells seem to never exceed 20% of the infiltrates (12), consistent with the present findings in rat oral mucosa.

In the mixed T cell population of CD4$^+$ and CD8$^+$ cells, approximately half of the cells expressed CD5 and/or TCR$\alpha\beta$. Generally, the relative proportions of the different subsets showed very little variability over time. The one exception was anti-CD45RB which stained only occasional cells at 2–6 h but a significant proportion at 24 h. CD45RB, detected by mAb OX22, defines a subset of T cells with the properties of "naive" cells, as opposed to previously activated "memory" cells which are CD45RB$^-$ (13). It is, however, not known to what extent CD45RB is expressed on cells other than lymphoid cells in the rat. Interestingly, in oral lichen planus which is a T cell dominated lesion, Walsh et al. (14) demonstrated CD45RA$^+$ dendritic non-keratinocytes and non-Langerhans cells within the epithelium. The expression of CD45RA in humans largely corresponds to rat CD45RB expression (15) but in the present study, no CD45RB$^+$ dendritic cells could be detected in the experimental lesions nor were any CD45RB$^+$ cells seen in normal mucosa. Furtermore, human monocytes are reported to be CD45R0$^+$ (i.e. CD45RB$^-$) (16). Based on these considerations and since current literature gives no indication that cellular activation may result in CD45RB induction in the rat, we conclude that the CD45RB$^+$ cells gradually migrate into our experimental lesions and comprise "naive" T cells. Thus, the early CH reaction seems to mainly involve memory T cells, of which at least some would be expected to be DNFB-specific. Human "memory" cells (CD29$^+$, CD44$^+$, CD45R0$^+$) have been shown to express elevated levels of adhesion molecules (CD2, CD11a/CD18, CD58) (1) indicating that they also have an increased propensity to migrate into the extravascular space. Minute stimuli in the early experimental CH reaction may thus be sufficient for the recruitment of migration-prone CD45RB$^-$ cells but not CD45RB$^+$ cells. Sterry (12) also found evidence in human skin of a selective migration of memory T over naive T cells, 1–2 days after antigen challenge at patch test sites.

The exclusive presence of CD45RB$^-$ T cells in the oral mucosa of animals that had been sensitized but not orally challenged raises the suspicion that memory type T cells may selectively migrate into the oral tissues prior to antigen challenge, conferring to this tissue a state of preparedness to react, once antigen challenge occurs. It is not known what could cause such a migration but supposedly there is a constant diapedesis of T cells, which has also been observed in normal skin (2). The situation may have some correlation to the clinical observations in humans, that the whole skin seems to be activated during patch testing (17). In states of increased incidence of migration-prone "memory" cells in the circulation, this spontaneous diapedesis may well manifest itself as T cell infiltrates in peripheral tissues. This has also been recorded by us (18) in experimental Hg-induced autoimmunity.

A large subset of the CD2$^+$ cells in the developing oral CH lacked CD5 as well as TCR$\alpha\beta$. As TCR$\gamma\delta^+$ cells have been shown to be CD5$^-$ or CD5^{dull} (19) the question arises if the CD2$^+$CD5$^-$ and the CD2$^+$ TCR$\alpha\beta^-$ cells are identical and if they are also TCR $\gamma\delta^+$. That this may be the case is indirectly supported by the findings of Hünig et al. (20), who showed that virtually all CD4$^+$ and approximately 95% of the CD8$^+$ lymph node T cells, as defined by the mAb OX52 which labels essentially the same population as OX19 (21), were also labelled with R73. A more precise evaluation of the role of TCR$\gamma\delta^+$ cells in the rat oral CH must however await the forthcoming of framework mAbs to the rat TCRγ or δ subunits.

A significant and consistent finding was that initial accumulation of CD2$^+$ cells always appeared at the epithelial-ct interface. The micro-anatomic component giving rise to this formation is unknown but may reflect T cell clustering with Langerhans cells (22, 23) known to be present in the rat oral mucosa at a density of approximately 160 cells/mm^2 (24). In contrast, although being potential antigen-presenting cells (25), ED1$^+$ cells do not seem to be involved initially as observed in the present study.

To summarize, we reason that the specificity of a CH reaction must be associated with the presence of antigen-specific (memory) T cells. In a presensitized individual, such cells would be expected to be over-represented in peripheral blood due to the clonal expansion induced by the sensitization procedure. Due to an increased migration-propensity, such memory cells may also be present in peripheral tissue prior to local antigen challenge. Seemingly contradictory is the view held by Herzog et al. (26), that initiation of T cell diapedesis depends on a T cell derived antigen-binding factor on mast cells. Mast cell involvement in early skin CH has also been

advocated by other authors, suggesting a neurogenic modulation of mast cell degranulation via substance P (27). By contrast, mast cell-deficient mice clearly produce CH to widely used contact sensitizers (28) with prominent, histologically verified mononuclear cell infiltrates at the site of challenge indistinguishable from control mice responses suggesting a critical role by the T cells themselves in the initiation of CH.

ACKNOWLEDGEMENTS

We greatfully acknowledge the skilful technical assistance of Mrs Ulla Samuelsson, the assistance with the statistical analysis by Prof Jan Lanke and the generous gift of mAb R73 by Dr Thomas Hünig, Munich, Germany. This study was supported by grants from the Swedish Medical Research Council No. 06872, the Swedish Dental Society and the Faculty of Dentistry, University of Lund.

REFERENCES

1. Sanders ME, Makgoba MW, et al. Human memory T lymphocytes express increased levels of three cell adhesion molecules (LFA-3, CD2, and LFA-1) and three other molecules (UCHL-1, CDw29, and Pgp-1) and have enhanced IFN-γ production. J Immunol 1988; 140: 1401–1407.
2. Bos JD, Zonneveld I, Das PK, et al. The skin immune system (SIS): distribution and immunophenotype of lymphocyte populations in normal skin. J Invest Dermatol 1987; 88: 569–573.
3. Groh V, Fabbi M, Hochstenbach F, Maziarz RT, Strominger JL. Double-negative ($CD4^-CD8^-$) lymphocytes bearing T-cell receptor α and β chains in normal human skin. Proc Natl Acad Sci USA 1989; 86: 5059–5063.
4. Janeway CA Jr, Jones B, Hayday A. Specificity and function of T cells bearing $\gamma\delta$ receptors. Immunol Today 1988; 9: 73–76.
5. Bos JD, Teunissen MBM, Cairo I, et al. T-cell receptor $\gamma\delta$ bearing cells in normal human skin. J Invest Dermatol 1990; 94: 37–42.
6. Warfvinge G, Larsson Å. Monoclonal antibody reactivity of oral mucosa in experimental contact hypersensitivity. In: Thivolet J, Schmitt D, eds. The Langerhans Cell. London, Paris: Colloque INSERM/John Libbey Eurotext Ltd, 1988; 172: 421–429.
7. Ahlfors EE, Larsson Å. Light microscopic study of experimental cell-mediated immunity in rat oral mucosa. Scand J Dent Res 1984; 92: 549–556.
8. Fisker AV, Stage I, Philipsen HP. Use of etorphine-acepromazine and diprenorphine in reversible neuroleptanalgesia of rats. Lab Animals 1982; 16: 109–113.
9. Li C-Y, Ziesmer SC, Lazcano-Villareal O. Use of azide and hydrogen peroxide as an inhibitor for endogenous peroxidase in the immunoperoxidase method. J Histochem Cytochem 1987; 35: 1457–1460.
10. van Loon LAJ, van Elsas PW, Bos JD, ten Harkel-Hagenaar HC, Krieg SR, Davidson CL. T-lymphocyte and Langerhans cell distribution in normal and allergically induced oral mucosa in contact with nickel-containing dental alloys. J Oral Pathol 1988; 17: 129–137.
11. Gawkrodger DJ, McVittie E, Carr MM, Ross JA, Hunter JAA. Phenotypic characterization of the early cellular responses in allergic and irritant contact dermatitis. Clin Exp Immunol 1986; 66: 590–598.
12. Sterry W, Bruhn S, Künne N, et al. Dominance of memory over naive T cells in contact dermatitis is due to differential tissue immigration. Br J Dermatol 1990; 123: 59–64.
13. Powrie F, Mason D. The MRC OX-22^- $CD4^+$ T cells that help B cells in secondary immune responses derive from naive precursors with the MRC OX-22^+ $CD4^+$ phenotype. J Exp Med 1989; 169: 653–662.
14. Walsh LJ, Tseng PW, Savage NW, Seymour GJ. Expression of CDw29 and CD45R antigens on epithelial cells in oral lichen planus. J Oral Pathol Med 1989; 18: 360–365.
15. Thomas ML. The leukocyte common antigen family. Ann Rev Immunol 1989; 7: 339–369.
16. Smith SH, Brown MH, Rowe D, Callard RE, Beverley PCL. Functional subsets of human helper-inducer cells defined by a new monoclonal antibody, UCHL1. Immunology 1986; 58: 63–70.
17. Grønhøj Larsen C, Ternowitz T, Grønhøj Larsen F, Thestrup Pedersen K. Epidermis and lymphocyte interactions during an allergic patch test reaction. J Invest Dermatol 1988; 90: 230–233.
18. Warfvinge G, Larsson Å. Spontaneous lymphocytic infiltrates of oral mucosa in $HgCl_2$ induced autoimmunity of BN rats: phenotypic characteristics and contact hypersensitivity to Hg. Reg Immunol 1991; 3: 88–96.
19. Groh V, Porcelli S, Fabbi M, et al. Human lymphocytes bearing T cell receptor (γ/δ) are phenotypically diverse and evenly distributed throughout the lymphoid system. J Exp Med 1989; 169: 1277–1294.
20. Hünig T, Wallny H-J, Hartley JK, Lawetzky A, Tiefenthaler G. A monoclonal antibody to a constant determinant of the rat T cell antigen receptor that induces T cell activation. J Exp Med 1989; 169: 73–86.
21. Robinson AP, Puklavec M, Mason DW. MRC OX-52: a rat T-cell antigen. Immunology 1986; 57: 527–531.
22. King PD, Katz DR. Mechanisms of dendritic cell function. Immunol Today 1990; 11: 206–211.
23. Shiohara T, Moriya N, Saizawa KM, Nagashima M. Role of Langerhans cells in epidermotropism of T cells. Arch Dermatol Res 1988; 280: 33–38.
24. Ahlfors EE, Larsson PÅ, Bergstresser PR. Langerhans cell surface densities in rat oral mucosa and human buccal mucosa. J Oral Pathol 1985; 14: 390–397.
25. Verschure PJ, van Noorden CJF, Dijkstra CD. Macrophages and dendritic cells during the early stages of antigen-induced arthritis in rats. Scand J Immunol 1989; 29: 371–381.
26. Herzog WR, Meade R, Pettinicchi A, et al. Nude mice produce a T-cell derived antigen-binding factor that mediates the early component of delayed-type hypersensitivity. J Immunol 1989; 142: 1803–1812.
27. Matis WL, Lavker RM, Murphy GF. Substance P induces the expression of an endothelial-leukocyte adhesion molecule by microvascular endothelium. J Invest Dermatol 1990; 94: 492–495.
28. Mekori YA, Chang JCC, Wershil BK, Galli SJ. Studies of the role of mast cells in contact sensitivity responses. Cell Immunol 1987; 109: 39–52.