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Phenotypic Changes in Langerhans' Cells after Infection with Arboviruses: a Role in the Immune Response to Epidermally Acquired Viral Infection?

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The role of Langerhans cells (LC) in the initiation of an immune response to a viral infection remains unclear. In vivo epidermal infection with the arboviruses West Nile virus and Semliki Forest virus significantly increased the expression of major histocompatibility complex class II antigens, CD54, and CD80 on LC. Thus, during an epidermally acquired viral infection, local LC appear to mature to a phenotype approximating that of lymphoid dendritic cells. This change may be important in the activation of naïve T cells and the subsequent clearance of viral infection.

On encountering an antigen in the epidermis, Langerhans cells (LC) migrate to the local lymph node, at which interaction with T lymphocytes results in the proliferation of antigen-specific T cells. This sequence of events is accompanied by phenotypic alterations, including an increase in the expression of major histocompatibility complex class I and II antigens (4, 22, 25) and the accessory and costimulatory molecules intracellular adhesion molecule-1–CD54, CD11a, CD18 (6, 21), and B7-1–CD80 (23), that enhance the T-cell-receptor-mediated activation of unprimed T cells. Few studies have investigated the role of the LC in the induction of cell-mediated immunity to a viral infection. We have developed an in vivo murine model for investigating the role of LC in the cell-mediated immune response to epidermally acquired viral infection, with a flavivirus, West Nile virus (WNV), and an alphavirus, Semliki Forest virus (SFV), being used. We report here the early in vivo upregulation of cell surface MHC antigens, CD54, and CD80 on LC, which is consistent with the maturation of LC to a lymphoid dendritic cell phenotype. We suggest that such changes are the result of local, virus-directed, and/or host-directed responses and may modulate T-cell activation during viral skin infection.

BALB/c mice were anesthetized with tribromoethanol (Avertin; Aldrich Chemical Co.) and injected intradermally in the ear with Vero cell-derived virus stocks (10) of WNV (5×10^5 PFU), SFV (5×10^5 PFU), or virus growth medium (mock infection), or the animal was left uninjected. At 8, 16, 24, or 48 h after the infection, epidermal cell suspensions derived from the ears were prepared as described previously (22) or tissue was fixed and stained with hematoxylin and eosin. Polyclonal anti-virus antibody labelling detected viral antigens in 10% of the cells examined at 24 h after infection. Staining with hematoxylin and eosin revealed marginating and occasionally extravasated neutrophils present within the deep dermis. However, there was never any involvement of the epidermis in this inflammatory response.

Flow cytometric analysis showed statistically significant increases in the cell surface expression of class I and class II MHC antigens on the whole epidermal cell population and the LC subpopulation, respectively, that were above those for the mock-infected and uninjected controls. Representative flow cytometric profiles of epidermal cell suspensions labelled for class I MHC antigens and zoomed profiles of the epidermal cell population labelled for class II MHC antigens after WNV infection are shown in Fig. 1. The profiles obtained following SFV infection were similar (data not shown). Fluorescence was measured with a FACScan apparatus (Becton Dickinson, San Jose, Calif.) with an argon laser set at 488 nm. A total of 5×10^4 gated cells from each labelled sample were analyzed, and dead cells were excluded on the bases of low forward scatter (cell size), low side scatter (membrane configuration), and propidium iodide staining. The class II MHC antigen-positive population was confirmed as LC by double labelling with dendritic cell family-specific antibody NLDC 145 (data not shown) and backgating to forward and side scatter, during which these populations fell into the characteristic high forward and low side scatter distributions and consistently constituted 2 to 5% of the total epidermal cell population (3). The amalgamated data in Fig. 1 show that infection with WNV induced a consistent time-dependent increase in class I MHC antigen expression on the epidermal cell population that was statistically significant at 16, 24, and 48 h after infection compared with mock-infected controls. Infection with SFV also resulted in an upregulation of class I MHC antigens on epidermal cells that was statistically significant at 24 and 48 h after infection. As also can be seen from Fig. 1, class II MHC antigen expression on the LC population was upregulated above the high level of constitutive expression after in vivo infection with WNV. Infection with SFV also increased class II MHC antigen expression on LC significantly. Variations in the level of class II expression were occasionally detected on keratinocytes after infection with either virus but were not statistically significant.

The increases in the expression of cell surface class I and II MHC antigens, both of which interact directly with the T-cell receptor, led us to investigate whether the expression of other molecules required for the stimulation of T cells was similarly

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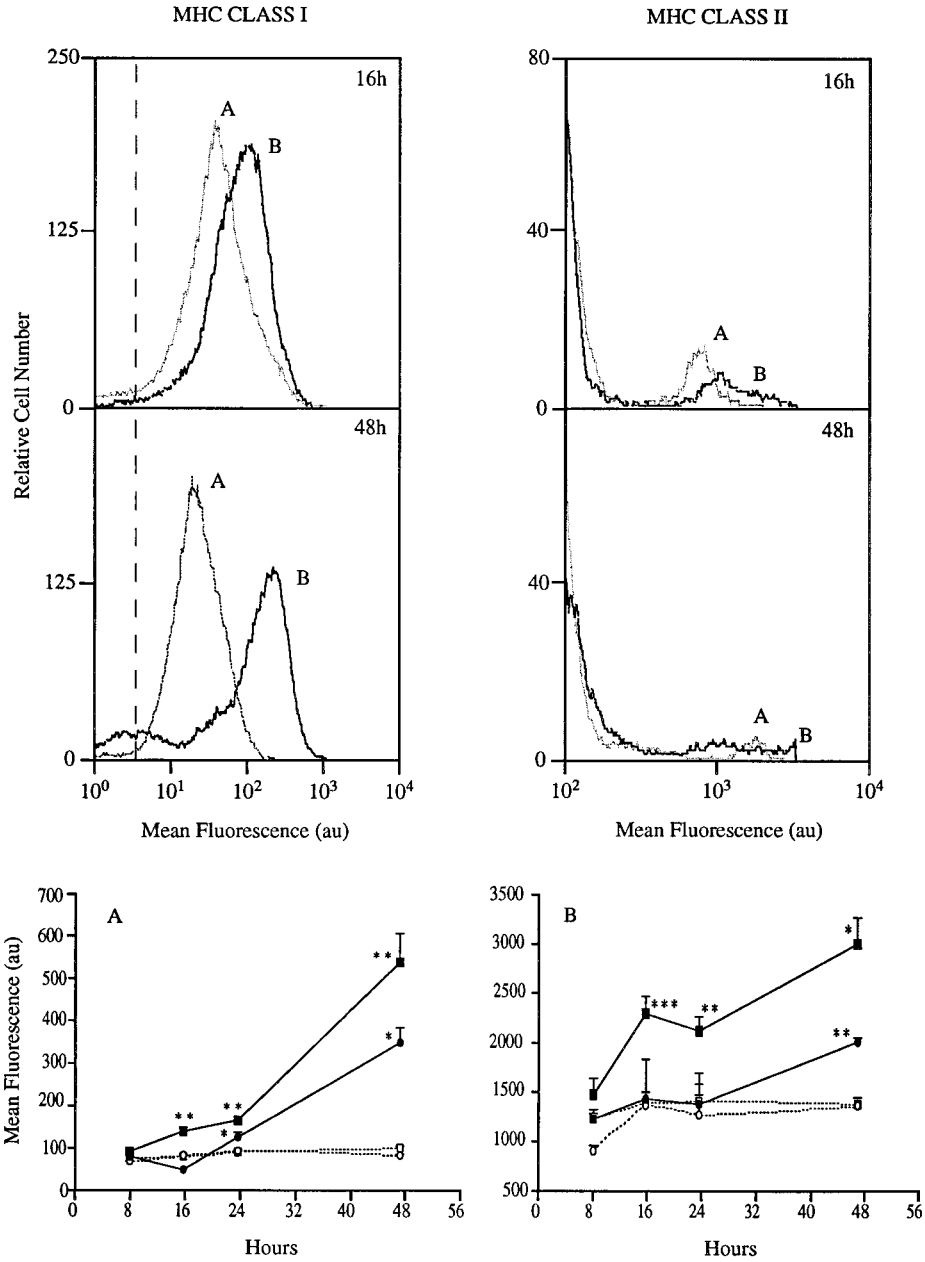

FIG. 1. Flow cytometric profiles illustrating the expression of class I MHC antigens (clone 34-5-8S [anti-*H-2D*^{d}]; American Type Culture Collection, Rockville, Md. [ATCC HB 102]) on the total epidermal cell population and class II MHC antigens (clone MK-D6 [anti-*I-A*^{d}] [ATCC HB 3]) on the LC population after in vivo mock infection (profile A) or WNV infection (profile B). The broken line represents the mean fluorescence of the isotype-matched antibody control (mouse immunoglobulin G2a isotype), which was omitted for clarity. Time-dependent increases in the expression of class I (A) and class II (B) MHC antigens on the surfaces of epidermal cells from uninjected (○), mock-infected (□), SFV-infected (●), and WNV-infected (■) mice are shown. The data represent the levels of antigen expression, as determined by mean fluorescence intensities plus the standard errors of the means, at each time point on all epidermal cells for class I MHC antigens and on the LC population for class II MHC antigens. Four groups of animals were used for each experiment, with two animals in each treatment group. Each datum at every time point represents the mean of at least three separate experiments. For statistical comparisons of two groups, an unpaired, two-tailed Student's *t* test was used. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.005$. au, arbitrary units.

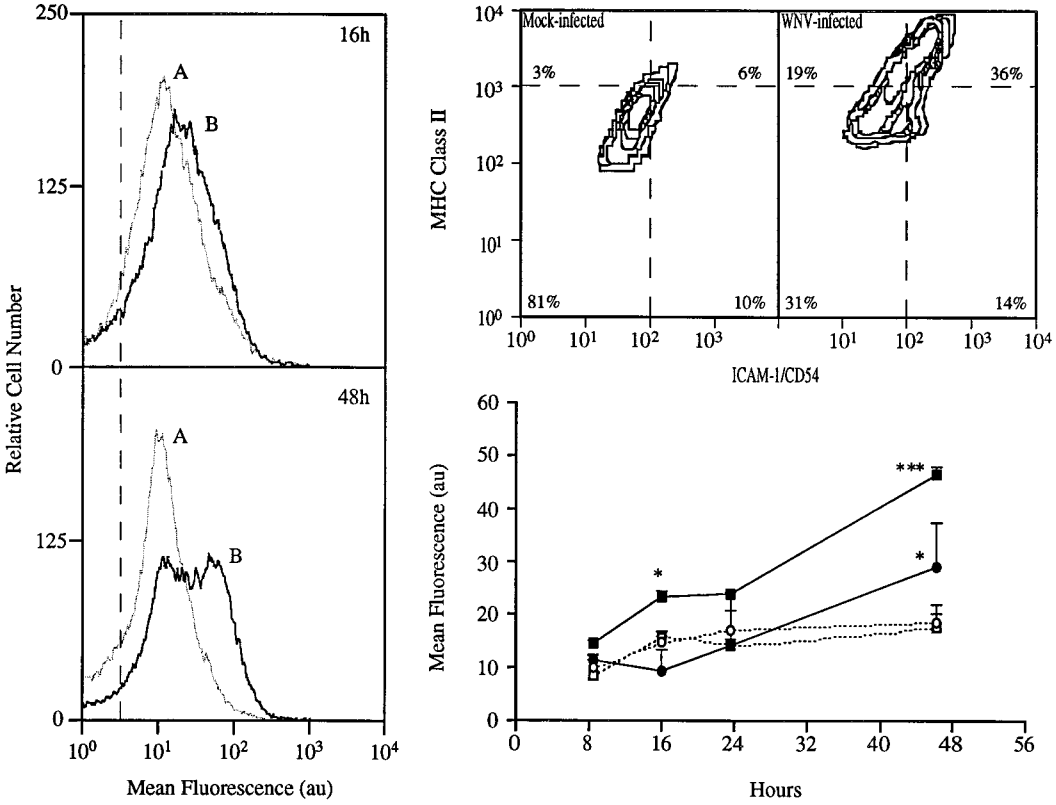

FIG. 2. Flow cytometric profiles illustrating the expression of CD54 (clone YN/1.7.4 [anti-CD54] [ATCC CRL 1878]) on the surfaces of epidermal cells after in vivo mock infection (profile A) or WNV infection (profile B) and two-color immunofluorescence analysis of LC (clones YN/1.7.4 and M5/114.15.2 [anti-$I\text{-}A^{b,d,q}$] [ATCC TIB 120]) 24 h after in vivo mock infection (A) or WNV infection (B). The time-dependent increases (with standard errors) in the expression of CD54 on the surfaces of epidermal cells from uninjected (○), mock-infected (□), SFV-infected (●), and WNV-infected (■) mice are shown. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.005$. au, arbitrary units; ICAM-1, intracellular adhesion molecule-1.

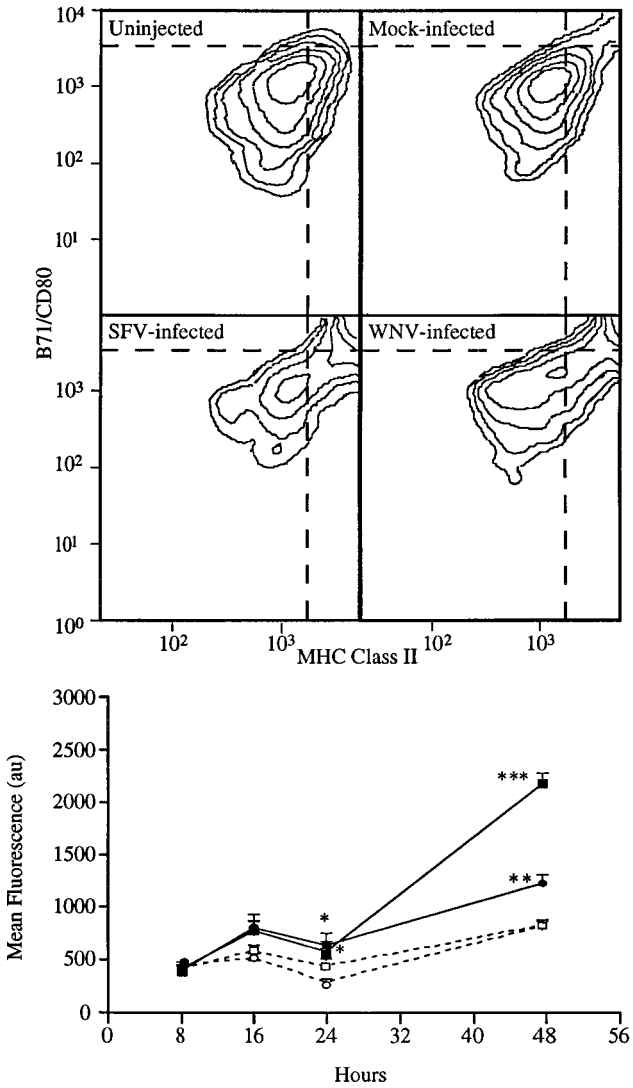

FIG. 3. Two-color immunofluorescence analysis of B7/CD80 (clone 1G10; Pharmingen, San Diego, Calif.) expression on LC at 48 h after noninjection, mock infection, or infection with SFV or WNV. The time-dependent increases (with standard errors) in the expression of CD80 on the surfaces of LC from uninjected (○), mock-infected (□), SFV-infected (●), and WNV-infected (■) mice are shown. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.005$. au, arbitrary units.

affected. It can be seen from Fig. 2 that CD54 expression was significantly upregulated above that of controls on approximately 10% of the epidermal cell population at 16 h after in vivo infection with WNV and on about 25% of the cell population by 48 h. Two-color immunofluorescence labelling revealed an increase in CD54 surface antigens in addition to the increase in class II MHC antigen expression on approximately 30% of the LC that was above that of the controls.

The effect of SFV or WNV infection on the expression of costimulatory molecule CD80 on LC was also determined by two-color immunolabelling (Fig. 3). Quadrants for the uninjected controls were set so that 0.4 and 0.5% of the gated LC population were found in the upper left (i.e., $CD80^{Hi}$) and upper right (i.e., CD80/MHC-II^{Hi}) quadrants, respectively. In LC from SFV- and WNV-infected epidermal cell populations, the percentages of LC in the upper right quadrants increased to 36.8 and 26.7%, respectively, of the gated LC population. Only LC which exhibited increased class II MHC antigen expression showed an increase in CD80 expression in the virus-infected animals, while in both cases, about 15% of the LC showed increased class II expression but did not show increased CD80 expression. Despite evidence that CD80 can be induced on keratinocytes (2), no induction of this molecule was detected on the keratinocyte population (data not shown).

To determine whether inflammatory cells immigrating into the dermis after infection could have contributed to the phenotypic changes we observed in the epidermal cells infected in vivo, isolated epidermal cell suspensions were infected in vitro with WNV or SFV or were mock infected. MHC class I antigen expression increased significantly on a subpopulation of epidermal cells, while class II expression also increased significantly on the LC population (Fig. 4). Alterations in CD54 and CD80 expression as a result of in vitro infection were inconclusive (data not shown). Interestingly, in vitro infection with SFV had no effect on the expression of any of the antigens.

Work from other laboratories has demonstrated increases in CD54 (21) and CD80 expression (2, 14) after treatment with phorbol esters, granulocyte-macrophage colony-stimulating factor, tumor necrosis factor alpha, or gamma interferon in vitro. To our knowledge, this is the first report of the in vivo upregulation of accessory or costimulatory molecules on LC in response to acute virus infection. The increases we observed in vivo were not temporally related to the infiltration of inflammatory cells within the dermis, in contrast to the observations of other studies that have described a correlation between the expression of ligands and the appearance of epidermotropic lymphocytes (17, 24), and these increases therefore suggest that the changes may have been a consequence of the production of keratinocyte- rather than lymphocyte-derived cytokines (1, 6, 8, 13, 19).

A further factor influencing these results may be that of flavivirus infection per se. Several viral infections, including those caused by measles virus and herpes simplex virus, are able to downregulate the expression of MHC class I surface antigens, thus making infected cells less susceptible to attack by cytotoxic T cells (9, 18). In contrast, infection with WNV results in an increase in the expression of the cell surface molecules involved in the activation of T cells (11, 15, 20). This functional upregulation is at least partially independent of the action of virus-induced interferons (5, 10, 12).

As WNV and SFV both gain entrance via the skin and are eradicated via the cell-mediated pathway (7, 16), we believe the use of this model is pertinent for the further study of the role of LC in the initiation of immune responses. The occurrence of phenotypic changes in the LC population suggest that these cells play an important role in the induction of the cell-

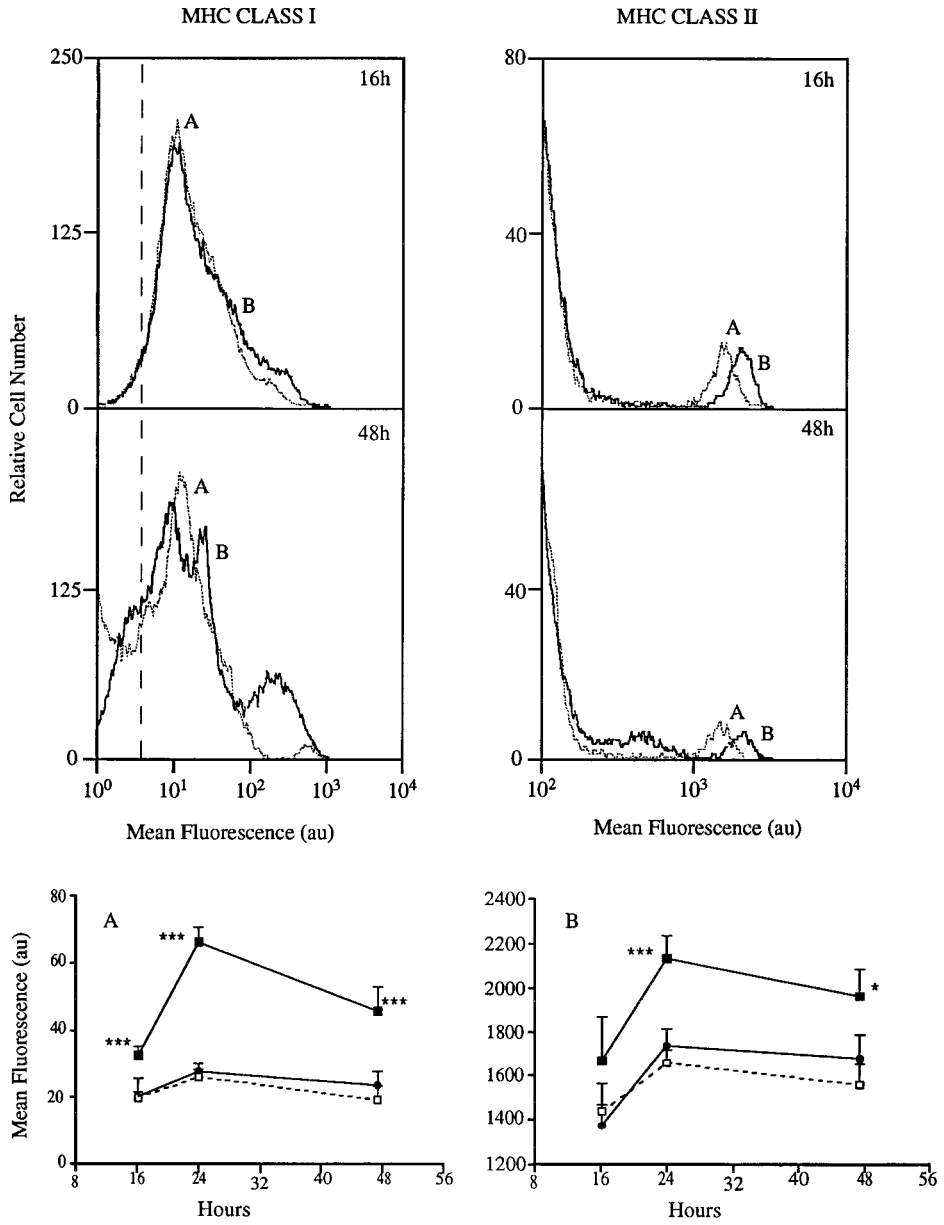

FIG. 4. Flow cytometric profiles illustrating the expression of class I MHC antigens on the total epidermal cell population and class II MHC antigens on the LC population after in vitro mock infection (profile A) or WNV infection (profile B). Time-dependent increases in the expression of class I (A) and class II (B) MHC antigens on the surfaces of epidermal cells from mock-infected (□), SFV-infected (●), and WNV-infected (■) mice are shown. The data represent the levels of antigen expression, as determined by mean fluorescence intensities plus the standard errors of the means, at each time point on all epidermal cells for class I MHC antigens and on the LC population for class II MHC antigens. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.005$. au, arbitrary units.

mediated antiviral immune response. Such changes, consistent with the differentiation to a mature, activated dendritic cell phenotype, could thus result in the local presentation of viral antigens to epidermotropic T cells and/or the heightened stimulation of T cells within the local lymph nodes. The acquisition of this phenotype, while perhaps being augmented directly by flavivirus infection, is likely, in the case of other epidermally transmitted viruses, to be the consequence of host-mediated responses that involve the production of a number of different cytokines in the local skin environment.

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