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T-Cell-Mediated Immunity to Lymphocytic Choriomeningitis Virus in β_2-Integrin (CD18)- and ICAM-1 (CD54)-Deficient Mice

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The T-cell response to lymphocytic choriomeningitis virus was studied in mice with deficient expression of β_2-integrins or ICAM-1. In such mice, the generation of virus-specific cytotoxic T lymphocytes was only slightly impaired and bystander activation was as extensive as that observed in wild-type mice. T-cell-mediated inflammation, assessed as primary footpad swelling and susceptibility to intracerebral infection, was slightly compromised only in β_2-integrin-deficient mice. However, adoptive immunization of mutant mice soon after local infection did reveal a reduced capacity to support the inflammatory reaction, indicating that under conditions of more limited immune activation both molecules do play a role in formation of the inflammatory exudate. Finally, virus control was found to be somewhat impaired in both mutant strains. In conclusion, our results indicate that although LFA-1–ICAM-1 interaction is important for certain aspects of the T-cell-mediated response to viruses, T-cell activation is surprisingly intact in these mutant mice, indicating extensive functional redundancy within cell interaction molecules.

Adhesion molecules are critically involved in all phases of the cell-mediated immune response (41). Most of our present knowledge concerning the function of specific adhesion molecules is derived from blocking studies utilizing monoclonal antibodies and soluble receptors. Such studies may, however, be misleading, since antibody binding to cell surface molecules may result in signalling effects due to cross-linking. In addition, the use of these reagents in vivo could lead to false-positive and -negative results arising from depletion of antigen-expressing cells or from inadequate penetration or amount of reagent. Therefore, mice carrying targeted mutations constitute an important tool for in vivo studies in this area of research (38).

One central adhesion molecule is LFA-1. LFA-1 belongs to the β_2-integrin family, the molecules of which are expressed on the cell membranes of leukocytes as $\alpha\beta$ heterodimers comprising a common β_2 chain (CD18) and a unique α chain (CD11a for LFA-1, CD11b for Mac-1, and CD11c for p150,95) (41). Known ligands for LFA-1 are ICAM-1, ICAM-2, and ICAM-3 (16, 41); ICAM-1 is also a counterreceptor for Mac-1 (19). ICAM-2 is constitutively expressed on most leukocytes and endothelial cells (17). ICAM-1 is normally found at a low level on a variety of cells, but expression is upregulated by inflammatory mediators, e.g., tumor necrosis factor alpha and gamma interferon (IFN-γ) (22, 24). This is true for endothelial cells (24), and ICAM-1 is therefore assumed to play an important role in leukocyte extravasation at inflammatory sites (27, 39). In addition, ICAM-1 and ICAM-3 have been reported to serve as costimulatory molecules on antigen-presenting cells (9, 16, 21, 40, 42, 44), and ICAM-1 has been reported to participate in cytotoxic T lymphocyte (CTL)-target cell conjugation (15, 18).

CD18 deficiency has been described for humans and is associated with severe recurrent bacterial infections (3, 7). In vitro, reduced T-cell proliferation and CTL activity have been demonstrated (6); however, no increased susceptibility to viral infections has been reported.

To study the role of β_2-integrins in the antiviral T-cell response, mice with induced mutations in the CD18 (46) and ICAM-1 (40) molecules were infected with lymphocytic choriomeningitis virus (LCMV). LCMV is a noncytolytic virus which does not induce inflammation in T-cell-deficient mice. However, in immunocompetent mice, marked $CD8^+$ T-cell activation results in an inflammatory reaction induced by $CD8^+$ effector cells (2, 20) and dominated by activated T cells ($CD8^+$ L-selectin$^+$ [low level] VLA-4$^+$ [high level] LFA-1$^+$ [high level] Mac-1$^+$ CD44$^+$ [high level]) and mononuclear phagocytes (4, 10, 11, 33). Moreover, in previous reports we have shown that antibodies to these molecules delay or totally inhibit the inflammatory reaction (5, 11, 33).

Virus-specific CTL activity in β_2-integrin- and ICAM-1-deficient mice. To evaluate the kinetics of CTL effector cell generation, mutant and wild-type mice (all obtained from the Jackson Laboratory, Bar Harbor, Maine) were infected intravenously (i.v.) with 10^3 50% lethal doses (LD_{50}) of LCMV (Traub strain [30]), and on days 7 and 8 postinfection (p.i.) splenocytes were tested for the ability to lyse virus-infected histocompatible MC57G cells in a standard ^{51}Cr release assay (43); these time points represent immediately prepeak and peak activities, respectively (12, 30). When analyzed on day 7 p.i., per-cell CTL activity was found to be reduced by about a factor of 2 in both mutant strains (Fig. 1), the difference being statistically significant at all effector-to-target ratios for β_2-integrin-deficient mice only. On day 8 p.i., CTL activity in ICAM-1-deficient mice was unimpaired whereas the induction of killing, on a per-cell basis, was reduced by about a factor of 4 in β_2-integrin-deficient mice. In this context it should be noted that the target cells were found by flow cytometry to lack expression of ICAM-1. Consequently, the reduced lysis mediated by effector cells from β_2-integrin-deficient mice cannot simply reflect reduced in vitro CTL-target conjugation.

Since differences in splenic-cell yields might significantly affect whether the above-presented dose-response curves are relevant to in vivo studies, we also determined the total num-

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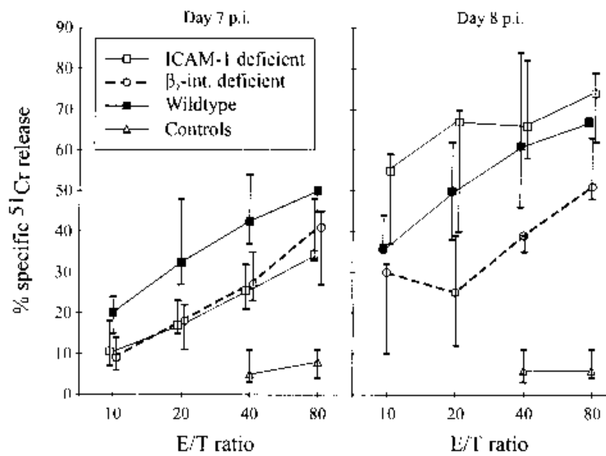

FIG. 1. LCMV-specific cytotoxicity in ICAM-1- and β_2-integrin-deficient mice 7 and 8 days after i.v. challenge with 1,000 LD_{50} of LCMV. Values are medians and ranges for three or four animals. E/T, effector to target.

ber of spleen cells on day 8 p.i. in mice of all three strains. Approximately the same number of spleen cells was found in all strains (ICAM-1 deficient, 8.6×10^7 cells; β_2-integrin deficient, 13.3×10^7 cells; and wild type, 11.9×10^7 cells [each a median value for three mice]), and we therefore concluded that per-spleen CTL activity essentially matched that found on a per-cell basis. Thus, on an overall level the generation of virus-specific CTLs was found to be surprisingly intact in the mutant mice, particularly in view of the markedly reduced capacity of splenocytes from the same strain of ICAM-1-deficient mice to function as stimulator cells in the mixed leukocyte culture (MLC) reaction (40). One interpretation of this difference is that while the MLC reaction relies on resting antigen-presenting cells for the triggering of the T-cell response, microbial challenge induces antigen-presenting-cell activation associated with upregulation of an array of surface molecules with costimulatory capacity. Accordingly, the lack of a single receptor-ligand interaction will not impair the immune response substantially under more natural conditions of stimulation. Indeed, a similar redundancy has been noted in previous studies involving, e.g., CD2-deficient mice (23).

Virus-induced polyclonal T-cell activation. Besides a potent virus-specific CTL response, LCMV infection is associated with marked bystander activation and proliferation of $CD8^+$ T cells (13). Thus, $CD8^+$ T cells constitute about 30 to 40% of all splenocytes around day 8 to 10 p.i., most of which are activated large granular T cells (4). The mechanism underlying this polyclonal activation is under debate, but in in vitro studies it has been demonstrated that the blocking of various adhesion molecules significantly inhibits nonspecific activation and proliferation of resting cells induced by antigen-activated T cells (8, 45). For this reason we found it relevant to evaluate the extent of bystander activation in LCMV-infected, β_2-integrin- and ICAM-1-deficient mice.

As a first parameter we evaluated, using flow cytometry, the fraction of $CD8^+$ T cells induced to enter the cell cycle and thus to undergo DNA synthesis. It has previously been found that this fraction peaks around day 7 p.i., at which time about one-third of $CD8^+$ T cells are in S or G_2/M phase (13). Therefore, on day 7 p.i. splenocytes from infected mutant and wild-type mice were surface labelled with anti-CD8a (Pharmingen, San Diego, Calif.), washed, and permeabilized with phosphate-buffered saline containing 0.03% saponin. Cells were then incubated for 30 min at room temperature with 4 μg of 7-amino actinomycin D (13) per ml, and the number of $CD8^+$ cells with a >2 N content of DNA was determined with a FACScan analyzer (Becton Dickinson, San Jose, Calif.). It was found that the same fraction of $CD8^+$ cells was progressing through the cell cycle irrespective of the mouse strain analyzed (Table 1).

Previous studies have shown that the clonal expansion of $CD8^+$ T cells in LCMV-infected mice is associated with the generation of T cells expressing the high-affinity form of the interleukin-2 receptor (IL-2R) and capable of proliferating in response to a low concentration of IL-2 in vitro (5). Splenocytes were therefore cultured for 24 h in the presence of 10 IU of recombinant murine IL-2 (Genzyme, Cambridge, Conn.) per ml, and proliferation was assessed by the addition of [^{3}H]thymidine during the last 6 h of incubation. From the results in Table 1 it is evident that cells from β_2-integrin-deficient as well as from ICAM-1-deficient mice responded as efficiently in this respect as did splenocytes from wild-type mice. Together, these results demonstrate that in the absence of β_2-integrin or ICAM-1 molecules, virtually identical polyclonal expansion is initiated in infected mice.

In normal mice, virus-induced polyclonal expansion results in the generation of a population of $CD8^+$ T cells characterized phenotypically by a high level of expression of several adhesion molecules, including LFA-1 and VLA-4 (4). Furthermore, we have found that these cells are functionally activated as evidenced, e.g., by the capacity to rapidly secrete large amounts of IFN-γ upon T-cell receptor signalling (13a). We therefore undertook to further evaluate LCMV-induced $CD8^+$ T-cell activation in the mutant mice by using two phenotypic markers of cellular activation (VLA-4 and CD71) and the production of IFN-γ as a functional marker.

Splenocytes from infected mutant and wild-type mice were surface labelled on day 7 p.i. with combinations of appropriate antibodies (all from Pharmingen) under standard conditions

TABLE 1. Polyclonal T-cell activation in LCMV-infected ICAM-1- and β_2-integrin-deficient mice[a,b]

Mouse type	% $CD8^+$ cells	% of $CD8^+$ cells that were:				IL-2 responsiveness[c]	IFN-γ production (ng/ml)[d]
		In S or G_2/M phase	$CD71^+$	$VLA\text{-}4^+$ high level	$LFA\text{-}1^+$ high level		
ICAM-1 deficient	27.5 (22.4–35.2)	24.3 (21.7–31.5)	34.1 (33.8–48.3)	62.5 (51.0–68.6)	64.3 (53.6–69.0)	37.1 (26.5–46.4)	38 (29–63)
β_2-integrin deficient	31.3 (27.3–33.7)	27.5 (24.2–29.8)	39.0 (34.4–43.9)	64.6 (62.2–68.5)	NA[e]	35.7 (33.2–40.2)	50 (25–60)
Wild type	31.4 (27.0–32.7)	32.2 (21.2–36.8)	45.2 (39.8–56.6)	74.8 (72.0–79.3)	69.8 (68.6–76.2)	32.6 (25.5–33.6)	49 (37–75)
Uninfected	12.1	5.6	14.7	9.0	17.9	1.7	<1

[a] Mice were infected with 1,000 LD_{50} of LCMV i.v. 7 or 8 days earlier.
[b] Values are medians (ranges) for three or four mice, except for uninfected mice. For uninfected mice, a typical result is presented.
[c] Stimulation index. A total of 2×10^5 cells were stimulated with 10 IU of murine IL-2 per ml for 24 h.
[d] 2×10^5 cells were stimulated with anti-CD3 for 6 h.
[e] NA, not applicable; see Fig. 2.

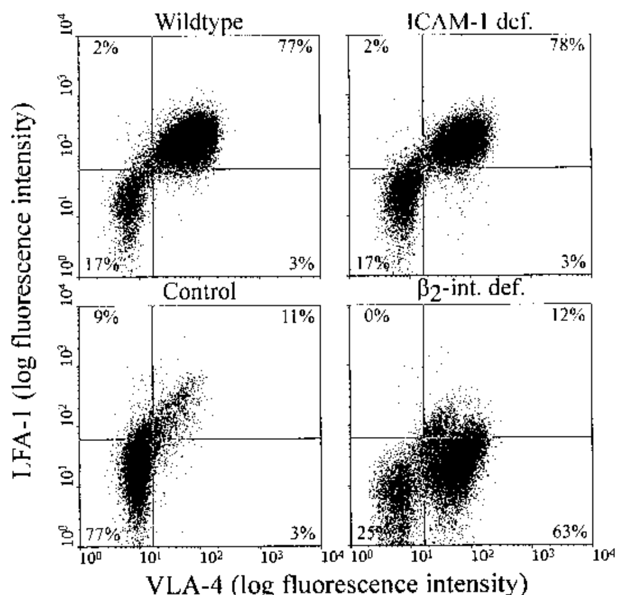

FIG. 2. Expression of adhesion molecules on CD8$^+$ T cells. Splenocytes from uninfected control mice and from primed ICAM-1-deficient, β_2-integrin-deficient, and wild-type mice were stained with anti-CD8, anti-VLA-4, and anti-LFA-1 on day 7 p.i. Gates were set for CD8$^+$ cells.

and analyzed with a FACScan analyzer and LYSYS II software (Becton Dickinson). Not surprisingly, only faint LFA-1 expression was observed in β_2-integrin-deficient mutant mice (Fig. 2), whereas comparable upregulation was found in ICAM-1-deficient and wild-type mice. More important, the upregulation of VLA-4 expression on activated CD8$^+$ T cells as well as the fraction of CD8$^+$ VLA-4$^+$ (high level) cells was found to be virtually identical in all three strains. Identical staining of CD8$^+$ cells was also observed in the three strains by using anti-CD71 (transferrin receptor, a marker for proliferating cells [32]) combined with anti-VLA-4 (Table 1).

To functionally evaluate effector T-cell differentiation, spleen cells from infected animals were stimulated with anti-CD3 for 6 h on day 7 p.i. and IFN-γ secretion into the supernatant was assayed by use of a sandwich enzyme-linked immunosorbent assay (Endogen, Cambridge, Mass.). By using this short-term stimulation regimen, in vivo-generated effector T cells are easily differentiated from naive cells and at the same time cytokine release from all activated T cells—not only those that are LCMV specific—is induced, which is important when trying to assess bystander activation (31, 37). As expected from the phenotypic analysis, it was found that T cells from both knockout strains were phenotypically activated and produced levels of IFN-γ of the same magnitude as those produced by T cells from wild-type mice (Table 1).

Together, these results demonstrate that the polyclonal expansion observed in all three mouse strains leads to the generation of similarly activated CD8$^+$ T cells, as judged by adhesion molecule expression and capacity to produce IFN-γ. Thus, bystander activation appears to be unimpaired in β_2-integrin- and ICAM-1-deficient mice. This seems to argue against two prevailing hypotheses concerning the mechanism of this profound T-cell activation. One is that bystander activation is a result of a nonspecific T-cell–T-cell interaction mediated by adhesion molecules (8, 45). From the present results it appears that at least LFA-1–ICAM-1 interaction is not critical. The other is that polyclonal T-cell expansion represents activation of T cells with a low affinity for virus-modified self (31, 37). If this were true, one would expect to find that the specific CTL response (representing predominantly cells with high-affinity T-cell receptors for virus-modified self) was less affected by the induced mutations than was bystander activation, since cells with T-cell receptors of low affinity would have to depend more on accessory molecules to become activated (21). Clearly, this prediction was not fulfilled; in ICAM-1-deficient mice neither type of response was substantially reduced, and in β_2-integrin-deficient mice only the virus-specific CTL response was slightly impaired. Therefore, the simplest explanation for the extensive T-cell activation seen in LCMV-infected mice seems to be a cytokine-driven recruitment of cells localized in the same microenvironment as activated antigen-specific cells. The failure to observe substantial polyclonal T-cell activation in LCMV-infected IL-2-deficient mice despite significant CTL induction (14) and the ability of IL-2 to induce similar phenotypic changes in cultured T cells (34) add credence to this hypothesis.

Virus clearance. A relevant measure of the capacity of CTL effectors to function in vivo is virus clearance in infected animals (28). Therefore, groups of five animals were infected i.v. with 1,000 LD_{50} of the Traub strain of LCMV, and 10 days later spleens, lungs, and livers were removed and virus titers were determined. From the results depicted in Fig. 3, it is evident that both β_2-integrin- and ICAM-1-deficient mice controlled the infection less efficiently than did wild-type controls, although virus titers were clearly lower than those in T-cell-deficient nude mice (data not shown). Moreover, it is notable that essentially the same hierarchy with regard to strain distribution was revealed in all organs, irrespective of whether adhesion molecules are thought to be involved in effector cell homing (liver and lungs) or not (spleen). Thus, in all organs β_2-integrin-deficient mice had the highest virus loads, followed by ICAM-1-deficient mice and then wild-type animals; however, the tendency was less pronounced for the lungs. Since a difference in the capacity to control the infection was found even in the spleen, which is the major site of effector T-cell generation in i.v.-infected mice, impaired effector cell homing is not in itself a sufficient explanation. While reduced or delayed CTL generation may be part of the reason for impaired virus control in β_2-integrin-deficient mice, it is unlikely to contribute significantly to this phenomenon in ICAM-1-deficient mice, which had at most a marginal impairment of their CTL response. Therefore, the reduced virus control observed in these mice strongly suggests that LFA-1–ICAM-1 interaction is required for optimal efficiency of CTLs in vivo. In this context it is of interest that ICAM-1 expression on splenocytes is markedly upregulated as a result of infection (data not shown).

Role of β_2-integrin and ICAM-1 in homing to infected organs. To study effector cell homing to infected areas in a more direct manner, two manifestations of LCMV-induced T-cell-mediated inflammation were analyzed: LCMV-induced fatal meningitis and footpad swelling induced by primary infection of the footpad (1, 47).

To test if a lack of β_2-integrin or ICAM-1 molecules would reduce the severity of LCMV-induced meningitis, groups of 11 mice were infected intracerebrally with 1,000 LD_{50} of LCMV. On day 7 p.i. three animals of each strain were sacrificed, and the number of inflammatory cells per microliter of cerebrospinal fluid was evaluated (4, 29). The remaining mice were used to determine susceptibility to lymphocytic choriomeningitis. When cellular infiltration was evaluated, high numbers of inflammatory cells were found in all three groups (Fig. 4); again, however, β_2-integrin-deficient mice tended to be slightly inferior, having the lowest median number of cells recruited to the

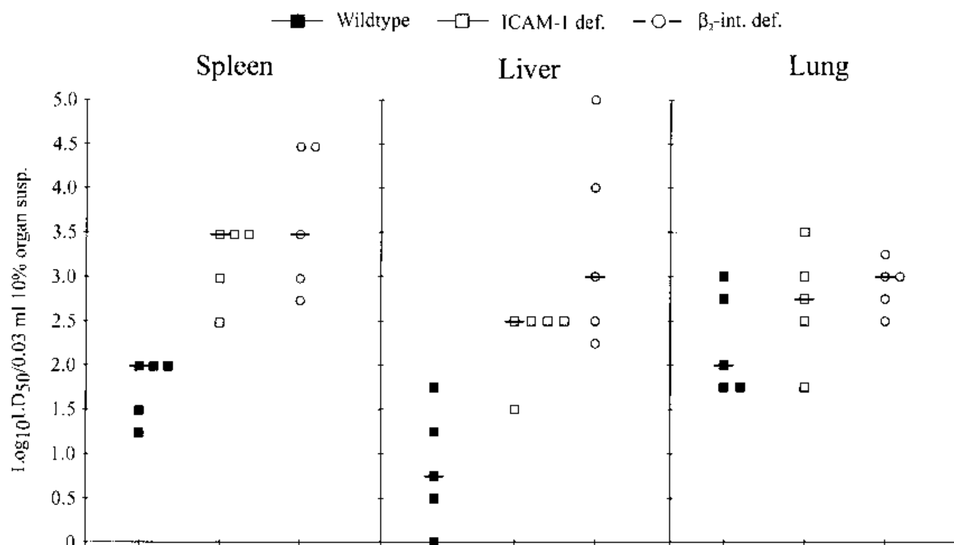

FIG. 3. Virus titers in spleens, livers, and lungs of ICAM-1-deficient, β_2-integrin-deficient, and wild-type mice. Mice were infected i.v. with 1,000 LD_{50} of LCMV, and on day 10 p.i. all mice were sacrificed and organ virus titers were determined. susp., suspension. Bars represent medians of groups.

brain. Parallel to this observation, it was found that in contrast to results for wild-type and ICAM-1-deficient mice, of which virtually all succumbed to lymphocytic choriomeningitis between days 7 and 9 p.i., the onset of disease was delayed in β_2-integrin-deficient mice, and although all displayed clinical symptoms, some survived the infection (Fig. 4).

Matching results were obtained when the primary footpad swelling reaction following local inoculation of the footpad with 10^3 LD_{50} of LCMV was assessed (Fig. 5). Again, the response in β_2-integrin-deficient mice was slightly but significantly delayed, whereas the response in ICAM-1-deficient mice followed the same kinetics as did the response in wild-type controls. Thus, on the whole, LCMV-induced T-cell-mediated inflammation was only marginally impaired in both of the mutant strains, and given that the induction of the specific T-cell response is somewhat compromised in β_2-integrin-deficient mice but not substantially impaired in ICAM-1-deficient mice, it cannot be excluded that it is primarily the generation of specific T cells which is rate limiting, not the capacity of effector cells to home to infected sites.

Reduced virus-specific delayed-type hypersensitivity reaction in β_2-integrin- and ICAM-1-deficient mice adoptively immunized with wild-type effector T cells. It is obvious from the above that the inability to separate the afferent and efferent phases of the immune response is a serious limitation in studies involving intact animals. Furthermore, inflammation is induced in animals with hyperactivated immune systems (cf. the abundance of activated T cells), in which the chance of functional redundancy is likely to be increased. Therefore, adoptive trans-

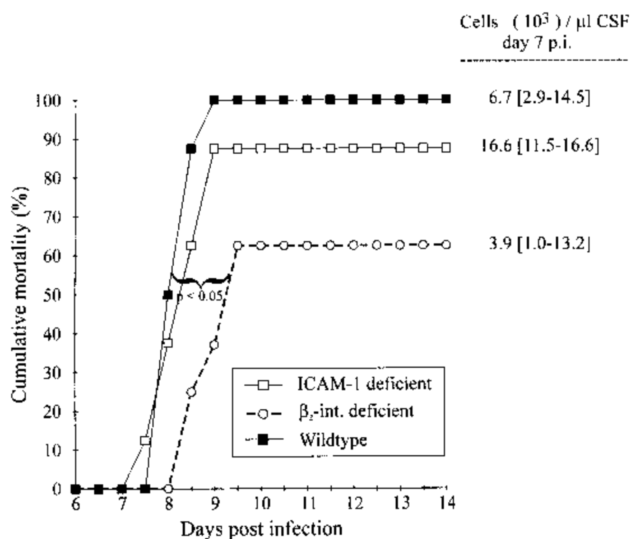

FIG. 4. Meningeal infiltration on day 7 p.i. (median [range] for three animals) and cumulative mortality in ICAM-1-deficient, β_2-integrin-deficient, and wild-type mice after intracerebral challenge with 1,000 LD_{50} of LCMV. Survival data are a compilation of results obtained in two experiments (n = 8). In uninfected mice meningeal infiltration did not exceed 100 cells per µl. CSF, cerebrospinal fluid.

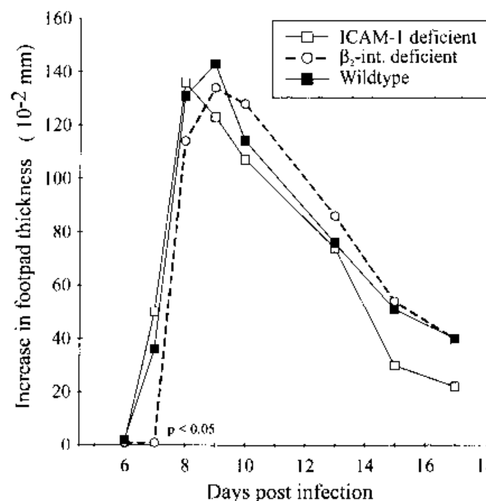

FIG. 5. Time course of footpad swelling in ICAM-1-deficient, β_2-integrin-deficient, and wild-type mice after footpad challenge with 1,000 LD_{50} of LCMV. Values are medians for four animals.

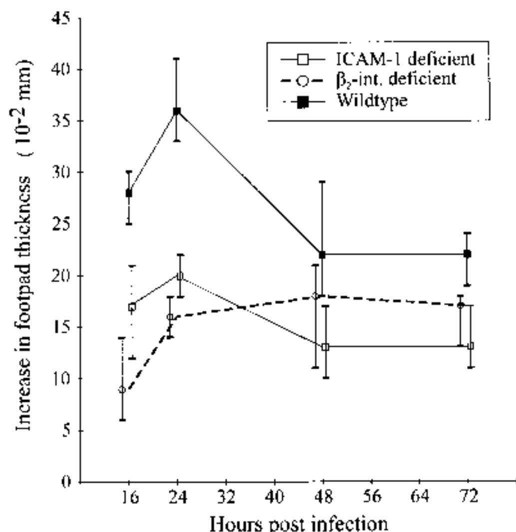

FIG. 6. Influence of ICAM-1 or β_2-integrin deficiency on ability to support elicitation of LCMV-specific delayed-type hypersensitivity. Recipients were infected with 10^7 LD_{50} of LCMV in the footpad and 4 h later received 5×10^7 LCMV-primed, adherent-cell-depleted wild-type donor splenocytes i.v. Donor cells were treated with mitomycin before transfer to prevent proliferation in the recipients. Values are medians and ranges for four animals.

fer of primed effector cells into newly infected recipients was carried out to more precisely determine the role of the LFA-1–ICAM-1 interaction in the formation of the inflammatory exudate. This approach was limited by the fact that effector T-cell generation was impaired in β_2-integrin-deficient mice, so that primed T cells from wild-type mice and from mice with an LFA-1 deficiency could not be directly compared for their homing capacities. However, primed T cells from wild-type mice were compared for the ability to home to the infected footpads of wild-type mice and of mice with no ICAM-1 expression (Fig. 6). In the latter case, the inflammatory reaction elicited by a bolus injection of effector T cells was significantly impaired, indicating that effector T cells, monocytes, or both used ICAM-1 as a ligand for extravasation. By giving adherent-cell-depleted splenocytes to β_2-integrin-deficient recipients, it could be directly demonstrated (Fig. 6) that monocytes—which in this setup had to be delivered by the recipient (33)—depended on β_2-integrins for extravasation. Thus, these results confirm and extend previous findings in which anti-LFA-1 and anti-Mac-1 antibodies were shown to inhibit the adoptive transfer of LCMV-specific delayed-type hypersensitivity (5, 33). The fact that little or no impairment of the inflammatory reaction is observed in intact ICAM-1-deficient mice whereas a significant effect is found in the adoptive model probably reflects the different experimental conditions obtained in the two situations and underscores the value of using both approaches. Thus, in the adoptive setup a rather limited number of effector cells are offered to the recipient and the individual cell probably gets few chances for interaction with the inflamed endothelium. Therefore, this approach is very sensitive in revealing molecules of importance. However, in intact animals the circulatory system is flooded with activated T cells, and even if the fraction of cells successfully interacting with the activated endothelium is somewhat reduced because of a lowered avidity, the sheer number of cells available will rapidly compensate for this and no effect will be observed. Thus, in contrast to results from the adoptive assay, those obtained for intact animals will provide highly relevant information about the complexity of the factors involved and will disclose any functional redundancy. Therefore, on the basis of this analysis we suggest that ICAM-1 is a relevant vascular ligand that contributes to the cellular interactions required for formation of the inflammatory exudate but that other vascular molecules (e.g., VCAM-1) provide the basis for sufficiently strong adhesion to ensure that the functional consequences of ICAM-1 deficiency are very limited in the natural setting, in which there is a surplus of activated leukocytes expressing several types of relevant receptors.

In conclusion, our study shows that although some impairment of the antiviral T-cell response is found in mice deficient in the expression of ICAM-1 or CD18, the impact of these defects is much less severe than expected from results obtained in model systems not representative of microbial challenge (9, 25, 26, 35, 36, 40, 46). Thus, the presence of β_2-integrins and ICAM-1 is not mandatory for the generation of an antiviral T-cell response and the efferent phase of the immune response is only slightly compromised. Therefore, our findings suggest that during challenge with an infectious agent, functional redundancy within cell interaction molecules will serve to minimize the consequences of interference with a single set of adhesion receptors. From an evolutionary point of view such redundancy is understandable, since viral infections are likely to have been a major factor in driving the development of a fail-safe T-cell system.

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