

Skin inflammation in RelB^{-/-} mice leads to defective immunity and impaired clearance of vaccinia virus

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Background: Atopic dermatitis (AD) is an inflammatory skin disorder occurring in genetically predisposed individuals with a systemic T_H2 bias. Atopic dermatitis patients exposed to the smallpox vaccine, vaccinia virus (VV), occasionally develop eczema vaccinatum (EV), an overwhelming and potentially lethal systemic infection with VV.

Objective: To establish a murine model of EV and examine the effects of skin inflammation on VV immunity.

Methods: The skin of RelB^{-/-} mice, like that of chronic AD lesions in humans, exhibits thickening, eosinophilic infiltration, hyperkeratosis, and acanthosis. RelB^{-/-} and wild-type (WT) control mice were infected with VV via skin scarification. Viral spread, cytokine levels, IgG2a responses and VV-specific T cells were measured.

Results: Cutaneously VV-infected RelB^{-/-}, but not WT mice, exhibited weight loss, markedly impaired systemic clearance of the virus and increased contiguous propagation from the inoculation site. This was associated with a dramatically impaired generation of IFN- γ -producing CD8⁺ vaccinia-specific T cells along with decreased secretion of IFN- γ by VV-stimulated splenocytes. The T_H2 cytokines—IL-4, IL-5, IL-13, and IL-10—on the other hand, were overproduced. When infected intraperitoneally, RelB^{-/-} mice generated robust T cell responses with good IFN- γ production.

Conclusion: Allergic inflammation in RelB^{-/-} mice is associated with dysregulated immunity to VV encountered via the skin. We speculate that susceptibility of AD patients to overwhelming vaccinia virus infection is similarly related to ineffective T cell responses.

Clinical implications: The susceptibility of patients with AD to EV following cutaneous contact with VV is related to ineffective antiviral immune responses. (J Allergy Clin Immunol 2007;119:671-9.)

Key words: Eczema vaccinatum, allergy, vaccinia virus, smallpox vaccination, viral response, cytotoxic T cells, T_H1/T_H2 cells

Rising concern regarding the use of variola virus, the etiologic agent of smallpox, as a biologic weapon has led to the resumption of smallpox immunization with live vaccinia virus (VV). Although historical experience supports the efficacy of immunization, it is associated with significant side effects. The most prevalent life-threatening reaction, eczema vaccinatum (EV), has an incidence of 38.5 cases per million vaccinees¹ and occurs only in patients with atopic dermatitis (AD)^{2,3} who experience a localized or potentially lethal systemic dissemination of the virus. The basis of the enhanced susceptibility to VV in AD patients is still not well understood.

Atopic dermatitis is a genetically determined chronic relapsing inflammatory skin disease, associated with striking abnormalities in systemic immune function, including elevated IgE and blood eosinophilia. The AD lesions are infiltrated with CD4 cells^{4,5} which in the acute phase predominantly secrete the T_H2 cytokines IL-4, IL-5, and IL-13, whereas in chronic lesions IFN- γ -producing T_H1 cells dominate.^{6,7}

The biology of AD has been studied in mice using either mutants⁸⁻¹⁴ or normal animals subjected to skin injury and allergen application.¹⁵ Because humans with AD have a genetic predisposition to the occurrence of diffuse skin inflammation, an optimal model would also exhibit genetically determined spontaneous allergic skin inflammation. One of the well characterized genetic models is the RelB^{-/-} strain which is uniformly affected by spontaneous dermatitis, hyperkeratosis, acanthosis, elevated serum IgE and skin infiltration with CD4 T cells and eosinophils.¹⁴ RelB^{-/-} skin contains increased levels of T_H2 cytokines IL-4 and IL-5 and, like chronic AD lesions in humans, elevated IFN- γ .¹⁴

RelB is a member of the NF κ B/Rel family of transcription factors, which are involved mainly in stress-induced, immune, and inflammatory responses. The 5 mammalian NF κ B/Rel family proteins are p65 (RelA), RelB, c-Rel, p50 (NF κ B1), and p52 (NF κ B2) and can be found in multiple combinations of homo- and heterodimers.¹⁶ The NF κ B/Rel family members influence T-helper cell differentiation; T_H1 cells express predominantly RelB, which promotes T-bet expression, whereas p50 and Bcl-3 dominate in T_H2 cells, where they can transactivate the GATA-3 promoter.¹⁷ Thus, the atopic skin inflammation

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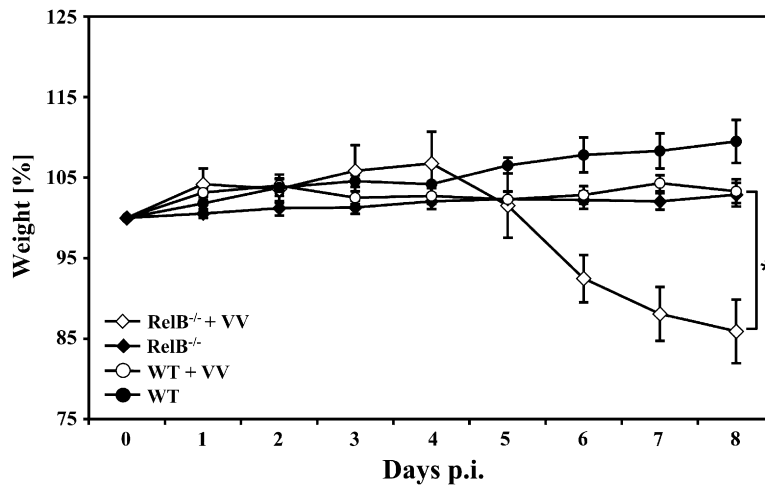


FIG 1. Effect of vaccinia infection on body weight of RelB^{-/-} and wild-type (WT) mice. RelB^{-/-} and WT mice were vaccinia virus (VV) infected via skin scarification with 1×10^7 plaque-forming units at the tail base or remained uninfected. Weights (normalized to weight on infection day 0, %) \pm SEM are shown for 8 days. Weight of VV-infected RelB^{-/-} mice was compared with VV-infected WT mice and statistically analyzed using 2-way ANOVA. * $P = .0082$; $n = 5$ per group). *p.i.*, Postinfection.

Abbreviations used

AD:	Atopic dermatitis
EV:	Eczema vaccinatum
i.p.:	Intraperitoneal
MHC:	Major histocompatibility complex
p.i.:	Postinfection
PFU:	Plaque-forming units
s.s.:	Skin scarification
TCR:	T-cell receptor
VV:	Vaccinia virus
WT:	Wild-type

displayed by RelB^{-/-} mice, like that in patients with AD, may arise both from exuberant skin inflammatory responses and from T_{H2}-polarized T cell and antibody responses.

We hypothesized that a tendency to allergic skin inflammation along with dysregulated adaptive immune responses as displayed by RelB^{-/-} mice might underlie the pathogenesis of EV. We considered that cutaneous infection of these mice with VV would provide an excellent model system in which to assess the pathogenesis of EV. To test this hypothesis, we infected RelB^{-/-} mice and WT controls with VV via skin scarification (s.s.) to mimic a smallpox immunization as applied in humans. Systemic illness and viral burden were assessed by monitoring mouse weight and quantifying numbers of viral genomes in a variety of tissues. Immune responses to VV were determined by measuring specific antibody titers and antiviral T cell responses. Our findings indicated that the atopic phenotype of RelB^{-/-} mice predisposes them to impaired immunity to VV and is associated with ineffective antiviral T_{H1} responses and induction of antiviral effector T cells.

METHODS

Virus source and expansion

The VV Western Reserve strain was obtained from American Type Culture Collection (ATCC) (VR-1354; Manassas, Va) and expanded and titered in CV1 cells (CCL-70; ATCC) by standard procedures.¹⁸

Animals and virus application

RelB^{-/-} mice on a C57BL/6 background were generated as described elsewhere.¹⁹ All experiments were carried out in accordance with Children's Hospital policies, and procedures and were reviewed by the Institutional Animal Care and Use Committee. For s.s., mice were anesthetized using avertin (2,2,2-tribromoethanol and tertiary amyl alcohol) and 10 μ L VV (1×10^7 plaque-forming units [PFU]) was inoculated with 30 superficial scratches at the tail base using a 27 $\frac{1}{2}$ -gauge needle. Alternatively, 100 μ L VV (2×10^6 PFU) was injected intraperitoneally.

Vaccinia-specific quantitative real-time PCR

DNA was prepared using the Qiagen DNeasy Kit (Qiagen, Valencia, Calif) according to the manufacturer's guidelines. Viral genomes were quantified by real-time PCR using primers specific for vaccinia ribonucleotide reductase (Vv14L) (see this article's Methods section in the Online Repository at www.jacionline.org for details).

In vitro cytokine synthesis by splenocytes

Single-cell suspensions of splenocytes were prepared and 1×10^6 splenocytes were incubated with 1×10^6 VV infected and uninfected irradiated stimulator splenocytes in 1 mL α -MEM complete medium. To prepare VV-specific stimulator cells, single-cell suspensions of splenocytes from naive mice were infected with VV (3 PFU per cell) or left uninfected (as control cells) and incubated overnight in α -MEM complete medium. Supernatants were collected at 24 h (for IFN- γ detection) and 72 h (for all other cytokines) and irradiated. Cytokine levels in supernatants were determined by ELISA following the manufacturer's instructions (BD Bioscience Pharmingen, San Jose, Calif).

Pentamer and intracellular IFN- γ staining

CD8⁺ T cells specific for the MHC class I restricted B8R₂₀₋₂₇ peptide were assayed by pentamer staining as described in this article's Online Repository at www.jacionline.org.

Statistical analyses

Differences in values between experimental groups were examined for significance with GraphPad Prism software using 2-way ANOVA (Fig 1) and the unpaired Student *t* test. Values are presented as means \pm SEMs. See Table E1 in this article's Online Repository for a listing of animal numbers used in each experimental group.

RESULTS

Weight loss in vaccinia-infected RelB^{-/-} mice

Weight loss is a common systemic manifestation of severe infection in mice. RelB^{-/-} and WT control mice were infected with VV at the tail base via s.s. The WT mice were observed to feed and groom normally and maintained their baseline weight (Fig 1). In contrast, RelB^{-/-} mice appeared listless and occasionally displayed a hunched posture beginning on day 6. Significant weight loss ($14.1 \pm 3.9\%$) occurred in vaccinia-infected RelB^{-/-} mice. These observations suggested a more severe systemic vaccinia infection in RelB^{-/-} mice.

Uncontrolled spread of VV in RelB^{-/-} mice

To assess the efficiency of systemic viral clearance in RelB^{-/-} and WT mice after cutaneous inoculation, we determined viral loads in a number of organs 8 days after infection. The RelB^{-/-} mice had dramatically (3-logs) higher viral burden in lung and liver ($9.3 \times 10^3 \pm 2.4 \times 10^3$ viral copies per μg total DNA in RelB^{-/-} lung vs. 6.4 ± 4.2 copies in WT lung and $1.2 \times 10^4 \pm 0.6 \times 10^4$ viral copies per μg total DNA in RelB^{-/-} liver vs. 12.3 ± 6.3 copies in WT liver) (Fig 2, A). Significantly impaired viral clearance was evident in kidney ($3.1 \times 10^3 \pm 1.5 \times 10^3$ viral copies per μg total DNA vs. 1.3 ± 0.9 copies in WT kidney). The kinetics of viral clearance were examined by measuring genomes in the liver, lung, and spleen at days 2, 4, and 8 after infection at the tail base. Whereas no viral copies could be detected in the organs of vaccinia-infected WT mice, RelB^{-/-} animals had increasing viral titers from day 2 to day 8 in liver, lung, and spleen (Fig 3).

Examination of viral loads at the inoculation site and contiguous skin sites revealed a variable defect in viral clearance in RelB^{-/-} animals. Eight days after infection, minimal VV ($6.8 \times 10^4 \pm 6.8 \times 10^4$ copies per μg total DNA) could be recovered from the inoculation site of 1 of the 15 WT mice studied (Fig 2, B). In contrast, variable but significant numbers of viral copies ($1.4 \times 10^6 \pm 0.4 \times 10^6$ per μg total DNA) were present in the inoculation site in 12 of the 13 RelB^{-/-} mice.

Analysis of skin samples 1.5 cm cephalad of the virus inoculation site at the tail base revealed occasionally defective viral clearance again only in RelB^{-/-} mice (Fig 2, C). High numbers of viral genomes ($1.9 \times 10^6 \pm 0.9 \times 10^6$ per μg total DNA) were recovered in 12 of the 13

RelB^{-/-} mice, but none from any of the 15 WT mice, indicating that the virus might spread from the inoculation site into contiguous regions only in the inflamed skin of the mutant animals. Taken together with our observations on organ viral loads, these findings indicate defects in viral clearance in the skin of the inoculation site in RelB^{-/-} mice followed by a propensity to spread to contiguous skin and ultimately the systemic organs.

Pattern of cytokines expressed by splenocytes of vaccinia-infected RelB^{-/-} mice

Because there is a marked cytokine dysregulation in the skin and systemically both in patients with AD and in RelB^{-/-} mice, we tested the hypothesis that an altered cytokine profile of vaccinia-specific CD4⁺ and CD8⁺ effector cells might underlie defective viral clearance. T cell cytokine responses were assessed in cultures of splenocytes stimulated with VV antigen-presenting cells. As expected, VV-stimulated WT splenocytes from vaccinia skin-infected animals mounted a robust IFN- γ response, secreting 61.6 ± 3.6 ng/mL IFN- γ into their supernatants (Fig 4), whereas splenocytes from vaccinia skin-infected RelB^{-/-} mice produced significantly lower IFN- γ levels (4.2 ± 1.9 ng/mL) (uninfected controls: 0.1 ± 0 ng/mL; data not shown). Polyclonal stimulation of T cells using anti-CD3 and -CD28 antibodies drove comparable levels of expression of IFN- γ by splenocytes derived from both RelB^{-/-} (2.0 ± 0.7 ng/mL) and WT (2.9 ± 1.4 ng/mL) mice (see Fig E1 in this article's Online Repository at www.jacionline.org). Taken together, the ability of CD3/CD28-stimulated cells from RelB^{-/-} mice to produce IFN- γ along with the fact that they have elevated IFN- γ levels in skin suggests that there is no intrinsic defect in IFN- γ gene expression in these mice. Yet it is clear from our findings that, paradoxically, the production of IFN- γ by virus-specific T cells in these animals is markedly suppressed.

Similarly, IL-2 was produced by vaccinia-stimulated splenocytes from infected WT (0.9 ± 0.2 ng/mL) but not RelB^{-/-} mice (0.01 ng/mL \pm 0.008) (Fig 4). In contrast to the attenuation of IFN- γ and IL-2 responses of virus-stimulated RelB^{-/-} splenocytes, the production of T_H2 cytokines was actually significantly enhanced compared with WT controls. Interleukin-13 (4.4 ± 0.7 ng/mL), IL-10 (3.7 ± 0.6 ng/mL), and IL-5 (15.1 ± 2.9 ng/mL) were produced at higher levels by RelB^{-/-} T-helper cells compared with RelB WT cells (IL-13 2.5 ± 0.2 ng/mL, IL-10 1.6 ± 0.2 ng/mL, and IL-5 1.6 ± 0.4 ng/mL).

Absent vaccinia-specific IgG2a antibody response in RelB^{-/-} mice

Virus-specific antibodies may be important effectors of viral clearance. In mice, measurement of immunoglobulin isotypes can also provide a window on the T_H1/T_H2 balance of T cell responses, because IFN- γ production is required for effective IgG2a production.²⁰ Vaccinia-specific IgG2a was first detected 8 days after infection (data not shown). Only vaccinia-infected WT control mice developed a vaccinia-specific IgG2a response (see Fig E2 in

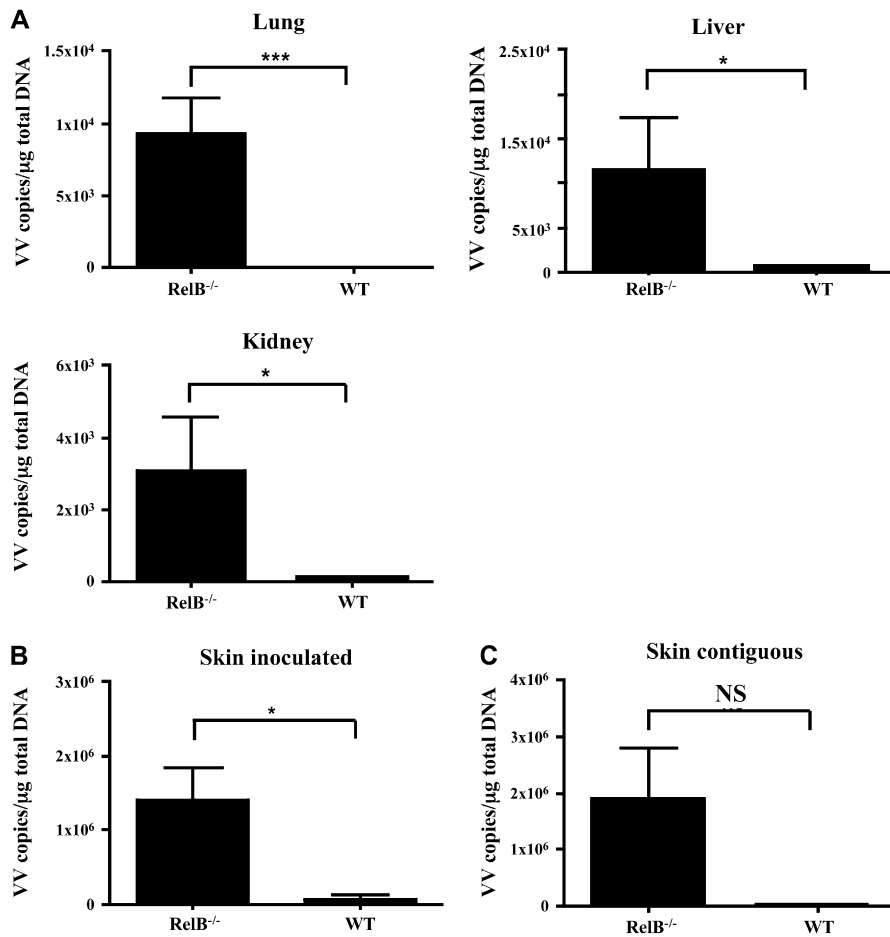


FIG 2. Viral burden in vaccinia virus (VV)-infected RelB^{-/-} and wild-type (WT) mice. Viral genome copy numbers \pm SEM are shown for lung, liver, and kidney (A), inoculation site (B), and contiguous skin (C) of VV-infected RelB^{-/-} and WT control mice 8 days after infection. DNA from uninfected control mice of each genotype was assayed to confirm the specificity of quantitative real-time PCR and did not contain any viral genome copies (data not shown). *** $P = .0008$; * $P < .05$; $n = 7-15$ for VV-infected mice. NS, Not statistically significant.

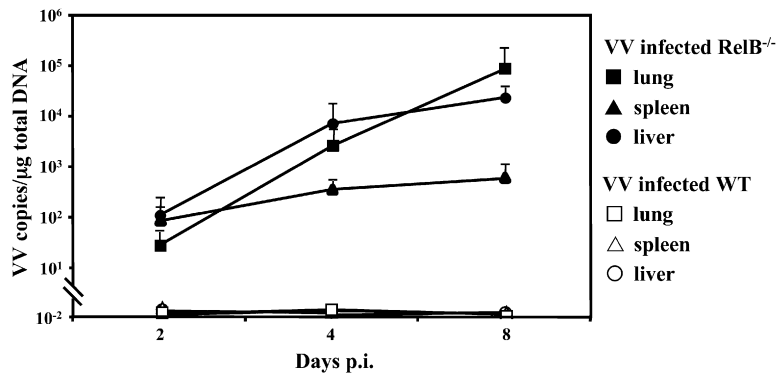


FIG 3. Systemic viral spread in vaccinia virus (VV)-infected RelB^{-/-} mice and wild-type (WT) control mice. Viral genome copy numbers \pm SEM in lung, liver, and spleen of VV-infected RelB^{-/-} and WT control mice are shown at postinfection (p.i.) days 2, 4, and 8 ($n = 2$, days 2 and 4; $n = 3$, day 8).

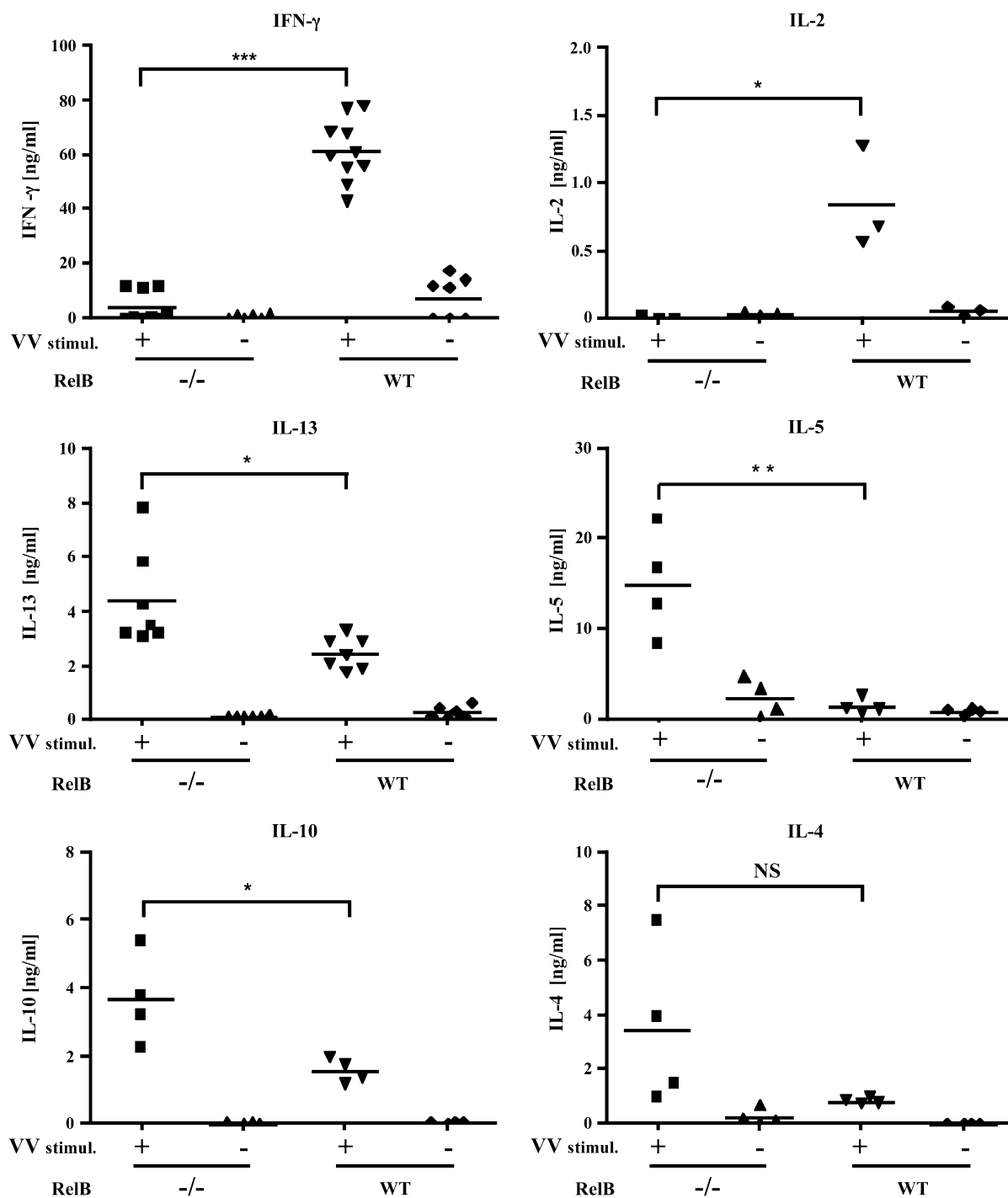


FIG 4. Cytokine production by vaccinia-stimulated splenocytes. Cytokine levels in the supernatants of splenocytes from vaccinia virus (VV)-infected RelB^{-/-} and wild-type (WT) control mice 8 days after infection, stimulated with VV-infected or uninfected splenocytes from naive WT mice. Data were compiled from analysis of 3 independent experiments. Splenocytes from uninfected mice did not produce cytokines upon VV stimulation (data not shown). **P* < .05; ***P* < .005; ****P* < .0001; *n* = 3-10.

this article's Online Repository at www.jacionline.org. The RelB^{-/-} mice had a markedly suppressed virus-specific IgG2a response. The decreased titers were not due to interference by VV virions, as confirmed by mixing

studies (data not shown). These findings identified a defective antivaccinia antibody response to vaccinia in RelB^{-/-} mice and corroborate our observation of suppressed vaccinia-specific IFN-γ production.

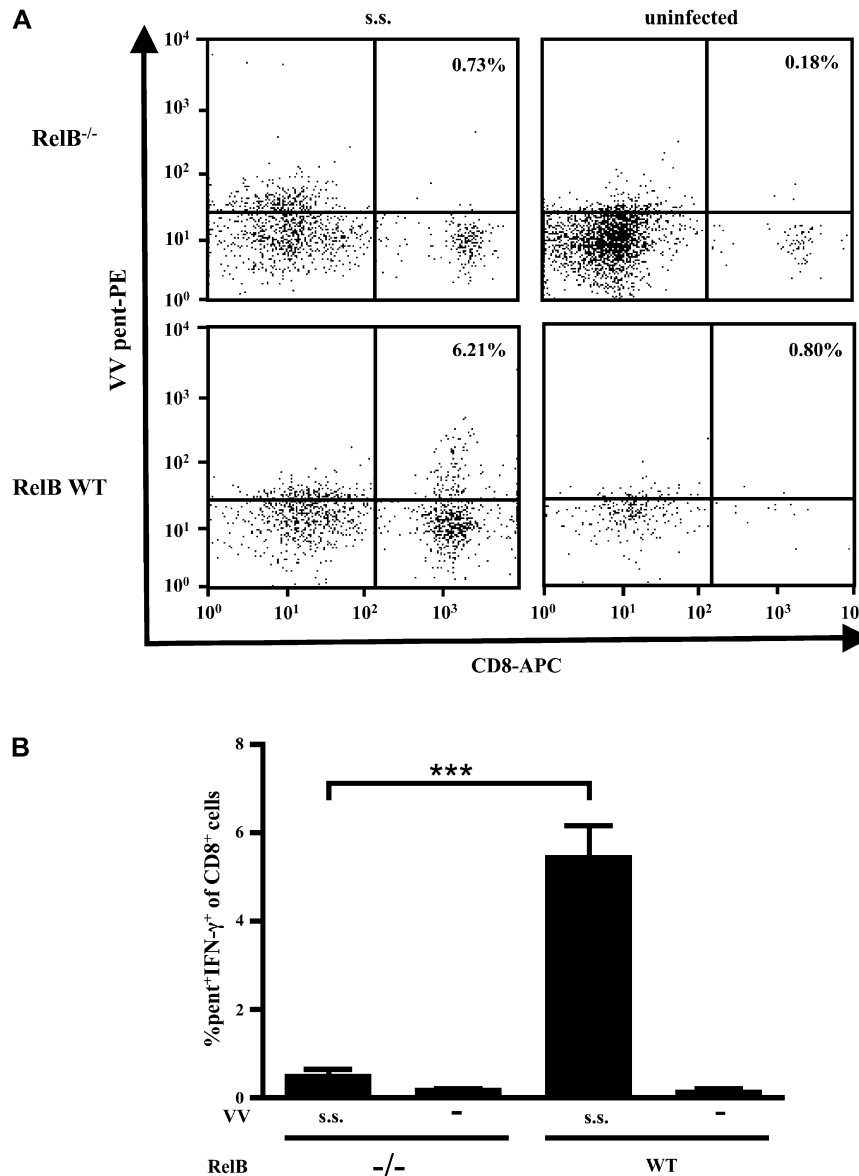


FIG 5. Vaccinia-specific CD8⁺ responses in RelB^{-/-} and wild-type (WT) mice. **A**, RelB^{-/-} and WT control mice were infected via skin scarification (s.s.), and 8 days after infection splenocytes were stained for CD8 and vaccinia virus (VV)-specific T-cell receptor (TCR; using B8R₂₀₋₂₇ MHC class I pentamers). Data were compiled from 3 independent experiments, and the dot plots shown are representative of 5 or more individual mice per group. **B**, Percentages of intracellular IFN-γ⁺ and VV-specific TCR-double positive splenocytes after stimulation with the VV-specific peptide B8R₂₀₋₂₇ (gated on CD8⁺ cells) ± SEM from VV-infected and uninfected RelB^{-/-} and WT control mice 8 days after infection via s.s. Data were compiled from 2 independent experiments. ****P* < .0001; n = 4 or more per group.

Absence of vaccinia-specific CD8⁺ effector cells in RelB^{-/-} mice infected via s.s.

Control of viral infections is achieved in part through the action of CD8⁺ T lymphocytes. We assayed the induction of this class of effector cells in infected mice using B8R₂₀₋₂₇ peptide-binding MHC class I pentamers. In WT mice, VV infection via s.s. resulted in CD8-positive and vaccinia-specific splenocytes (5.7 ± 0.32%; representative examples shown in Fig 5, A). In contrast,

CD8/pentamer-positive cells were absent from the spleens of RelB^{-/-} animals VV infected via s.s. (0.46 ± 0.32%; *P* < .0001). Consistently, we found only in WT mice VV-specific IFN-γ producing effector cells (5.5 ± 0.66%), and their almost complete absence in RelB^{-/-} mice (0.54 ± 0.12%; *P* < .0001; Fig 5, B). These findings provide evidence that, in addition to lacking IFN-γ positive T-helper cells and IFN-γ-dependent IgG2a responses, RelB^{-/-} mice also fail to generate a vaccinia-specific

IFN- γ -producing CD8⁺ T cell response after VV infection via skin scarification. The absence of all three of these immune effector mechanisms along with the pathologic increase in T_{H2} cytokine responses may underlie the impaired viral clearance of these animals when the route of infection is the skin.

Effect of infection route on immune responses of RelB^{-/-} mice

To establish whether the abnormal immunity of RelB^{-/-} mice is specifically associated with virus entry through inflamed skin, we infected RelB^{-/-} mice intraperitoneally and assayed the generation of VV-specific T cells. Eight days after i.p. VV injection, we observed a robust induction of pentamer-positive T cells in RelB^{-/-} mice (6.15 ± 3.01%, a similar level to that observed following i.p. infection of WT mice [3.0 ± 0.45%]; Fig 6, A). In contrast, s.s., which elicited a vigorous specific T cell response in WT mice, gave rise to only a minimal (<1%) population of virus-specific T cells in RelB^{-/-} mutants. Upon peptide stimulation, IFN- γ was induced in pentamer⁺ CD8⁺ T cells of intraperitoneally infected RelB^{-/-} (3.45 ± 1.59%) and WT mice (1.89 ± 0.25%) (data not shown). In addition, splenocytes from intraperitoneally infected RelB^{-/-} and WT mice cultured with VV antigen-presenting cells produced similar IFN- γ levels (1.32 ± 0.25 ng/mL and 1.06 ± 0.45 ng/mL, respectively; Fig 6, B), whereas only background amounts of IFN- γ were recovered in the supernatants of peptide-stimulated splenocytes from cutaneously sensitized RelB^{-/-} mice. The difference in IFN- γ secretion by splenocytes from intraperitoneally versus s.s.-infected RelB^{-/-} mice was significant ($P = .0004$). Splenocytes cultured with uninfected stimulator cells produced only background levels of IFN- γ (data not shown). By far the most robust response in this assay was observed in splenocytes from WT mice infected via the skin, indicating that cutaneous virus encounter is normally potently T_{H1} inducing. Thus, VV infection by i.p. injection, unlike s.s., generated antiviral effector T cell responses in RelB^{-/-} mice. This observation indicates a crucial role of normal skin in inducing protective immunity in WT mice and of allergic skin inflammation in inducing dysfunctional vaccinia responses in RelB^{-/-} mice.

DISCUSSION

The immunologic basis of enhanced susceptibility to VV in AD patients is not well understood. Immunity to vaccinia involves both adaptive and innate mechanisms. Innate responses include viral activation of Toll-like receptor and induction of type I interferons and antimicrobial peptides. Recently, it was observed that VV induces the expression of the antimicrobial peptide LL-37 in human skin.²¹ Adaptive immunity is also critical for viral clearance and involves T and B lymphocyte mechanisms. We hypothesized that these adaptive mechanisms might be altered in the setting of a T_{H2} cytokine profile and examined this possibility using RelB^{-/-} mice, which,

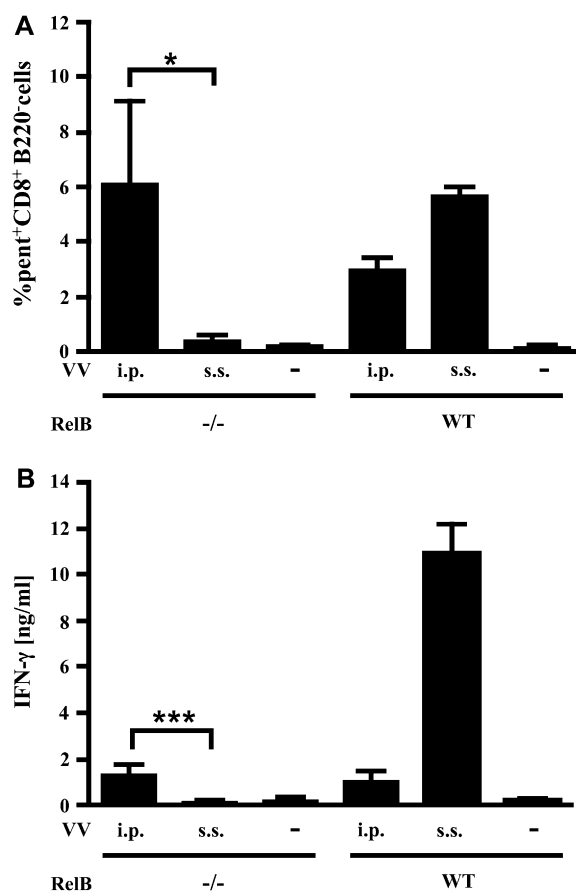


FIG 6. Vaccinia-specific T-cell responses in RelB^{-/-} and wild-type (WT) mice after i.p. infection. **A**, Eight days after infection via skin scarification (s.s.) or i.p. injection splenocytes from RelB^{-/-} and WT control mice were stained for CD8 and vaccinia virus (VV)-specific T-cell receptor (using B8R₂₀₋₂₇ MHC class I pentamers) and analyzed by flow cytometry. Data were compiled from 3 independent experiments. * $P = .0366$; $n = 6$ or more per group. **B**, IFN- γ levels in the supernatants of splenocytes from intraperitoneally or s.s. VV-infected or uninfected RelB^{-/-} and WT control mice 8 days after infection, cultured with VV-infected splenocytes from naïve WT mice. Cytokines were determined by ELISA 24 hours after stimulation. Data were compiled from 3 independent experiments. *** $P = .004$; $n = 6$ or more per group.

like humans with AD, spontaneously develop diffuse allergic skin inflammation. We infected these mice with VV via s.s. mimicking smallpox immunization in AD patients and observed for evidence of impaired viral clearance and altered immune responses.

The first evidence of increased VV-associated morbidity was accelerated weight loss indicative of systemic illness (Fig 1). This was associated with markedly elevated viral burdens in the viscera as well as in the inoculated and contiguous skin in VV-infected RelB^{-/-} mice (Fig 2). In addition, kinetic analysis confirmed steadily increasing viral genome copy numbers in lung, liver, and spleen from postinfection (p.i.) day 2 to 8 in RelB^{-/-} but not in WT mice, indicating a defect in viral clearance (Fig 3).

Because markedly dysregulated cytokine expression occurs in the skin and systemically both in patients with

AD and in $RelB^{-/-}$ mice, we tested the hypothesis that altered cytokine production by vaccinia-specific $CD4^{+}$ and $CD8^{+}$ effector cells and consequent abnormalities in the profile of the adaptive T_H1/T_H2 response might underlie defective viral clearance. Our findings support this hypothesis; we observed that antigen-stimulated splenocytes from $RelB^{-/-}$ mice VV infected via s.s. did not produce IFN- γ (Fig 4). Interferon- γ is absolutely necessary for effective viral clearance.^{22,23} This cytokine confers potent antiviral properties on infected cells, as shown by its inhibition of VV replication in mouse macrophages via induced nitric oxide.^{24,25} In addition, IFN- γ drives T_H1 -biased inflammatory immune responses and has many effects on immune cell physiology, including the activation of dendritic cells and macrophages and stimulation of specific cytotoxic immunity by promoting MHC class I and II expression.²⁶

The absence of an antiviral IFN- γ response was also reflected in the inability of $RelB^{-/-}$ mice to produce VV-specific IgG2a (see Fig E2 in this article's Online Repository at www.jacionline.org). Furthermore, VV-specific IFN- γ -producing $CD8^{+}$ T cells, which are known to be important immune effector cells in viral immunity, also failed to be induced in the $RelB^{-/-}$ mice infected via s.s. (Fig 5). The defect in IFN- γ production and induction of virus-specific effector cells is related to the route of viral entry. $RelB^{-/-}$ mice infected intraperitoneally generated IFN- γ -producing T cells at frequencies comparable with WT mice (Fig 6). In addition, we previously observed that these animals produce large amounts of IFN- γ in the skin at baseline¹⁴ and have elevated plasma IFN- γ following *Listeria* infection.²⁷ In the present study we also observed that, when polyclonally stimulated, $RelB^{-/-}$ splenocytes have the capacity to produce IFN- γ at levels comparable with those made by WT splenocytes (see Fig E1 in this article's Online Repository at www.jacionline.org). Thus, it appears that there is a very specific defect in the induction of IFN- γ -producing T_H1 cells, IgG2a, and virus specific IFN- γ -producing $CD8$ cells if initial virus encounter occurs in inflamed skin.

In contrast with their defect in T_H1 cytokine production, VV-specific splenocytes from $RelB^{-/-}$ mice displayed a robust T_H2 cytokine response, producing IL-4, which is known to induce and sustain T_H2 cells, and IL-10, which indirectly reduces cytokine production by T_H1 cells and down-regulates MHC class II molecules.²⁸ In addition to inhibiting the expansion of IFN- γ -producing T_H1 effector cells, T_H2 cytokines are known to attenuate cytotoxic T lymphocyte activity *in vivo* and to inhibit viral clearance.²⁹⁻³¹ Vaccinia virus infection in the presence of IL-4 coexpression has been reported to result in down-regulation of T_H1 cytokines, reduction of cytolytic activity, and impairment of viral clearance.³¹⁻³³ Interleukin-4 and IL-13 were also shown to up-regulate VV replication in human skin and to down-regulate the antimicrobial peptide expression in a signal transducer and activator of transcription 6-dependent manner.²¹ Furthermore, it was reported that mice lacking the T-box expressed in T-cells (T-bet) transcription factor, which have a T_H2 bias and

enhanced IL-4 expression, are more susceptible to primary VV infection.³⁴ Therefore, elevated levels of IL-4 in $RelB^{-/-}$ skin may exert similar effects.

Developing a murine model of eczema vaccinatum and defining its immunologic pathogenesis offers important opportunities. The $RelB^{-/-}$ system provides a potentially useful screening tool for the assessment of novel vaccines, particularly with regard to their tendency to spread in the skin and organs of mice affected, like patients with AD, both by spontaneously arising diffuse skin inflammation and by T_H2 polarized systemic immune responses. Taken together with studies performed in normal animals, such a model system could provide an opportunity to evaluate new vaccines with respect to both optimizing their immunogenicity and at the same time attenuate their tendency toward local and systemic spread. It will be critical in future studies to identify the viral and host factors which may be exploited to induce strong protective $CD8^{+}$ IFN- γ^{+} T cell responses even in the setting of AD.

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