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Immunology

A mechanism for the inhibition of fever by a virus

(interleukin-1β/cytokine receptor/virus pathogenesis/vaccinia virus)

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ABSTRACT **Poxviruses encode proteins that block the activity of cytokines. Here we show that the study of such virulence factors can contribute to our understanding of not only virus pathogenesis but also the physiological role of cytokines. Fever is a nonspecific response to infection that contributes to host defense. Several cytokines induce an elevation of body temperature when injected into animals, but in naturally occurring fever it has been difficult to show that any cytokine has a critical role. We describe the first example of the suppression of fever by a virus and the molecular mechanism leading to it. Several vaccinia virus strains including smallpox vaccines express soluble interleukin 1 (IL-1) receptors, which bind IL-1β but not IL-1α. These viruses prevent the febrile response in infected mice, whereas strains that naturally or through genetic engineering lack the receptor induce fever. Repair of the defective IL-1β inhibitor in the smallpox vaccine Copenhagen, a more virulent virus than the widely used vaccine strains Wyeth and Lister, suppresses fever and attenuates the disease. The vaccinia-induced fever was inhibited with antibodies to IL-1β. These findings provide strong evidence that IL-1β, and not other cytokines, is the major endogenous pyrogen in a poxvirus infection.**

Fever is an ancient nonspecific systemic response to infection, which is conserved in both invertebrates and vertebrates (1). Temperature elevation during fever constitutes a component of host defense that increases host survival and enhances different arms of the inflammatory and immune responses, such as T-cell proliferation and differentiation, secretion of interferons (IFNs), neutrophil migration, and the neutralizing capacity of antibodies (1–3). Fever may also have detrimental effects in some circumstances (1, 2).

Fever induced after infection or inoculation of exogenous pyrogens, such as lipopolysaccharide, is mediated by release of soluble proteins that act directly or indirectly on the central nervous system. Candidate endogenous pyrogens include the cytokines interleukin-1α (IL-1α), IL-1β, tumor necrosis factor-α (TNF-α), TNF-β (lymphotoxin-α), IL-6, IFN-α, and IFN-γ (1). These molecules induce body temperature elevation when inoculated into experimental animals but their contribution to fever under physiological conditions has been more difficult to establish, partly due to the lack of experimental models for naturally occurring fever. Dissecting the role of cytokines in fever is further complicated because they have pleiotropic effects, interact with each other at multiple levels, and can increase pathology following their overproduction.

Poxviruses are large cytoplasmic DNA viruses that normally produce an acute infection in the host (4, 5). Vaccinia virus (VV) was the vaccine used to eradicate smallpox, a devastating human disease produced by variola virus (6). Different vaccine strains induced different reactions to vaccination and frequencies of postvaccinial complications, but the reasons are unknown (6).

Poxviruses encode many proteins that interfere with host immune functions (7, 8), such as soluble cytokine receptors that blockade cytokines and modulate virus virulence (9). The VV IL-1β receptor (vIL-1βR) is encoded by gene B15R in the Western Reserve (WR) strain (10), and binds IL-1β with high affinity, but not IL-1α or IL-1 receptor antagonist (11). Inactivation of the B15R gene from VV WR decreases virus virulence in mice after intracranial injection (12) but enhances virus virulence when administered intranasally (11). These contradictory results might be explained by either deletion mutant virus having additional mutations affecting virus virulence or, alternatively, may reflect the properties of IL-1β, a cytokine that induces beneficial effects but is detrimental when produced in large amounts (13). Since a natural route of poxvirus infections is the respiratory tract (4), the vIL-1βR may function to prevent the adverse effects of excessive IL-1β.

Here we report that the WR vIL-1βR prevents the febrile response in intranasally infected mice. In this model, smallpox vaccine strains lacking the vIL-1βR induced fever, and repair of the nonfunctional vIL-1βR in strain Copenhagen prevented fever and attenuated virus virulence.

MATERIALS AND METHODS

Cells and Viruses. The growth conditions for BS-C-1 and TK^-143B cells and the sources of VV strains have been described (14).

Reagents. Radioiodinated mouse or human recombinant IL-1β (80–180 μCi/μg) was obtained from DuPont/New England Nuclear. In competition experiments with human ^{125}I-IL-1β we used unlabeled human and mouse recombinant IL-1β (2×10^8 units/mg) from R & D Systems. In competition experiments with mouse ^{125}I-IL-1β we used unlabeled mouse recombinant IL-1α (8×10^6 units/mg) and IL-1β (3.5×10^5 units/mg) from Genzyme. The hamster monoclonal antibody B122 that neutralizes mouse IL-1β was provided by D. Chaplin (Washington University School of Medicine, St. Louis) (15).

Construction of Recombinant VVs. The VV WR deletion mutant lacking B15R (vΔB15R) has been described (11). A plaque-purified wild-type virus vAA8 (vB15Rwt) and a revertant virus vAA9 (vB15Rrev) were constructed by transient dominant selection (16, 17). Plasmid pAA20 was derived from pSJH7 (18) by insertion of a 2854-bp *Sal*I–*Bam*HI DNA fragment excised from pAA1 (11) and containing B15R and flanking regions. Plasmid pAA20 was used for reinsertion of the WR B15R gene into the endogenous locus of vΔB15R and the corresponding locus of the Copenhagen strain. Following the transient dominant selection procedure, a plaque-purified Copenhagen virus (vAA10 or vCOP-vIL-1βR$^-$) and a Copen-

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Abbreviations: dpi, days postinfection; IFN, interferon; IL, interleukin; pfu, plaque forming units; TNF, tumor necrosis factor; vIL-1βR, vaccinia virus IL-1β receptor; VV, vaccinia virus; WR, Western Reserve.

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hagen strain expressing the B15R protein (vAA11 or vCOP-vIL-1βR$^+$) were isolated. The genomic structure of the recombinant viruses was confirmed by Southern blotting and PCR analysis.

Animals. Female BALB/c mice (5–6 weeks old) were housed at 20–22°C with light from 8 a.m. to 8 p.m. Mice were infected intranasally with purified virus (11). Rectal temperature was determined each day (9–11 a.m.) with a mouse rectal probe (Harvard Apparatus) and a digital thermometer. The change in body temperature was calculated by subtracting the baseline temperature of each group, determined during 6–8 days before infection, from each subsequent time point. Body temperature was also determined with the Electronic Laboratory Animal Monitoring System from BioMedic Data Systems (Maywood, NJ). Microchip battery-free transponders (14 mm × 2.2 mm, 120 mg) were implanted subcutaneously in the dorsal thoracic area with a sterile needle. Temperature readings were collected with a DAS-5004 Pocket Scanner. Mice were also weighed daily and monitored for signs of illness (ruffled fur, arched back, and reduced mobility). Virus infectivity in blood (collected by intracardiac puncture) and various organs was determined by plaque assay on TK$^-$143B cells (19).

Assays for Soluble IL-1βRs and IL-1β. Binding assays for soluble IL-1βRs were performed with ^{125}I-IL-1β as described (11). Plasma and organ homogenates (50 μl) were centrifuged at 13,000 rpm for 5 min in a microcentrifuge before assay. Levels of mouse plasma IL-1β were determined with the InterTest-1βX mouse IL-1β ELISA kit (Genzyme).

RESULTS

Fever Is Induced in Mice Infected with VV WR Lacking the vIL-1βR. A VV WR lacking a functional B15R (vΔB15R) was previously constructed by transient dominant selection (11). A plaque-purified WR virus (vB15Rwt) derived from the same intermediate virus as vΔB15R and, therefore, genetically closely related to vΔB15R, was used here. In addition, a virus revertant (vB15Rrev) was constructed by reinsertion of B15R into vΔB15R by transient dominant selection. The expression of vIL-1βRs by these viruses was as predicted (not shown).

The pathogenicity of these viruses was tested in intranasally infected BALB/c mice, which produces an extensive respiratory infection and virus dissemination to other organs. Infection of groups of 10 mice with doses ranging from 5×10^3 to 10^7 plaque forming units (pfu) of WR, vB15Rwt, vΔB15R, and vB15Rrev produced similar numbers of mortalities, and animals infected with vΔB15R showed accelerated weight loss and signs of illness (not shown), confirming previous observations (11). To investigate this further, we measured the body temperature of infected mice, since fever is one of the systemic responses affected by IL-1 (Fig. 1). Surprisingly, intranasal inoculation with 2.5×10^3 or 7.5×10^3 pfu of VV wild-type (vB15Rwt) did not induce fever but reduced body temperature by 0.5–1°C. By 6 or 7 days postinfection (dpi), the body temperature decreased dramatically, with temperatures as low as 26–30°C at high virus doses, which coincided with severe signs of illness and weight loss. After day 10, surviving animals started to recover and body temperature and weight returned to normal levels by 22 dpi. In contrast, animals infected with vΔB15R developed fever during the first 5–6 dpi (Fig. 1). Later, these animals also developed hypothermia and recovered from infection at the same rate as those infected with vB15Rwt. Reinsertion of the B15R gene into vΔB15R (vB15Rrev) restored the wild-type phenotype, demonstrating that vIL-1βR expression was wholly responsible for the suppression of fever. The difference of 1–2°C in body temperature 1–6 dpi with viruses that do or do not express the vIL-1βR was statistically highly significant (Fig. 1). The extent of fever in infected mice housed at 20–22°C was similar to that reported in studies with mice housed at 30°C (20, 21). The accelerated appearance of signs of illness and weight loss in mice infected with both doses of vΔB15R (Fig. 1; not shown) were also

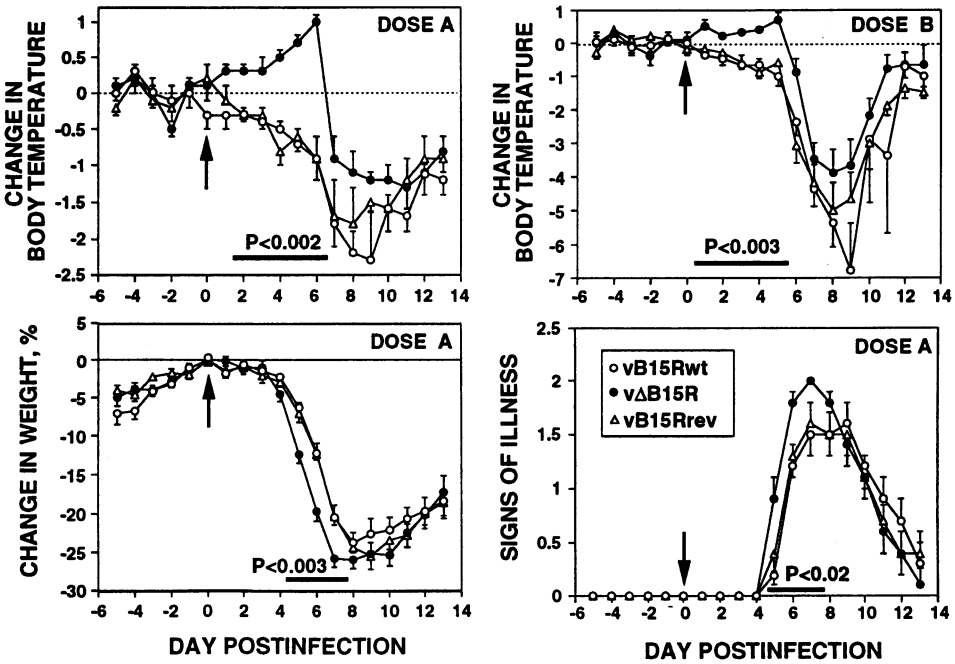

FIG. 1. Infection of mice with WR VVs, which do or do not express the vIL-1βR. Groups of 10 mice were intranasally infected with 2.5×10^3 (dose A) or 7.5×10^3 (dose B) pfu of vB15Rwt (○), vΔB15R (●), or vB15Rrev (△). The mean change in rectal temperature ± SEM and the basal temperature (broken line), which ranged from 35.9 to 36.3°C, are shown. The mean change in weight ± SEM is expressed as the percentage of the mean weight of the group immediately prior to infection (solid line). Signs of illness were scored from 0 to 4, and the mean value ± SEM of each group is shown. The arrow indicates the day of virus inoculation. The horizontal bars indicate those days in which the difference between vΔB15R and both vB15Rwt and vB15Rrev were statistically significant when analyzed by Student's t tests, and the mean P value is shown, with maximum values of $P < 0.009$ (temperature and weight) and $P < 0.05$ (signs of illness). The number of mice per group that died or were sacrificed because of severe infection were 0–1 or 4–8 for dose A or B, respectively.

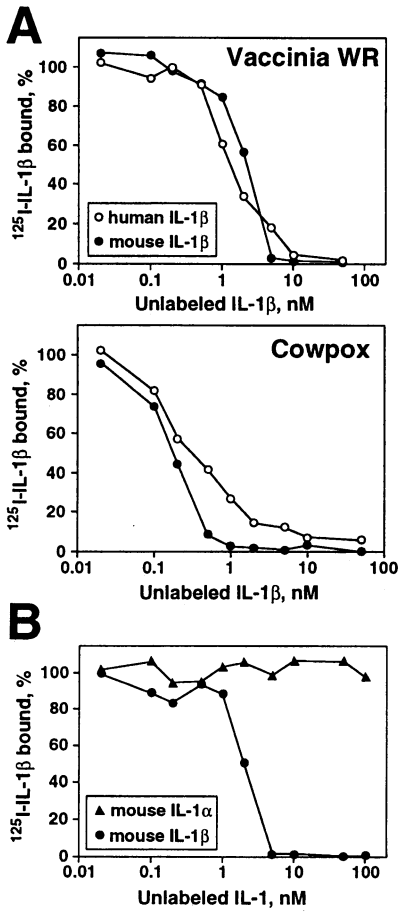

FIG. 2. Binding characteristics of the vIL-1βR. (*A*) Binding of human and mouse IL-1β to the vIL-1βR. Medium from 7×10^5 cells infected with VV WR or cowpox was incubated with 100 pM of human ^{125}I-IL-1β in the presence of the indicated concentrations of unlabeled human (○) or mouse (●) IL-1β, and the radioactivity bound to the soluble receptor was determined. Binding is expressed as the percentage of the binding occurring in the absence of competitor (10,517 and 4,073 cpm for VV WR and cowpox, respectively). (*B*) Binding of mouse IL-1α and IL-1β to the vIL-1βR. Medium from 7×10^5 cells infected with VV WR was incubated with 100 pM of mouse ^{125}I-IL-1β in the presence of increasing concentrations of unlabeled mouse IL-1α (▲) or IL-1β (●), and the radioactivity bound to the soluble receptor determined. Binding is expressed as the percentage of the binding occurring in the absence of competitor (18,200 cpm).

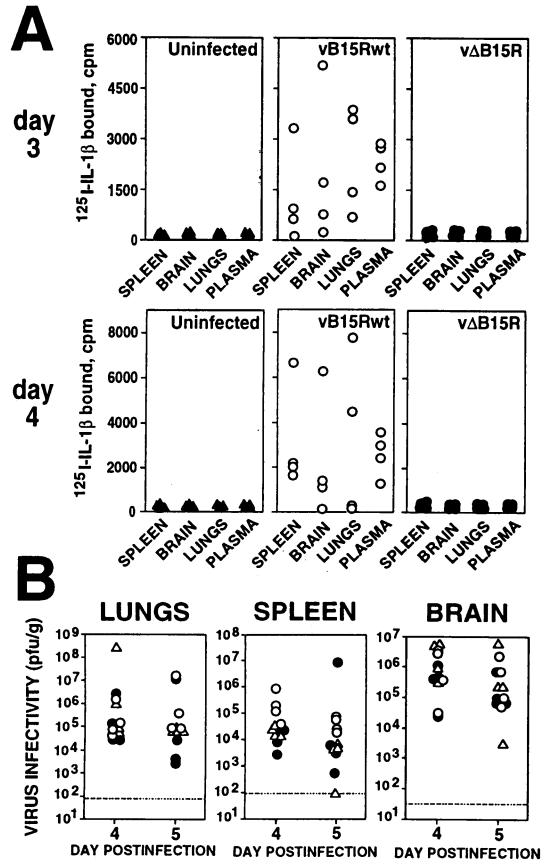

FIG. 3. Expression of soluble IL-1βRs and virus replication in infected mice. (*A*) Soluble IL-1β binding activity in VV-infected mice. Groups of four mice uninfected (▲) or intranasally infected with 10^7 pfu of vB15Rwt (○) or vΔB15R (●) were sacrificed at 3 and 4 dpi. Fifty microliters of plasma or homogenates from lung, spleen, or brain were incubated with 150 pM human ^{125}I-IL-1β in a soluble binding assay. The bound radioactivity is shown. (*B*) Virus replication in organs of infected mice. Groups of four mice were intranasally infected with 10^7 pfu of vB15Rwt (○), vΔB15R (●), or vB15Rrev (△) and sacrificed on the indicated dpi. The virus infectivity present in homogenates from lungs, spleen, or brain was determined in cell monolayers. The broken line indicates the detection limit of the plaque assay.

statistically highly significant and the full restoration of the wild-type phenotype in vB15Rrev demonstrated that the vIL-1βR was entirely responsible for these differences.

Infection of mice with VV WR and vB15Rwt gave similar results in terms of body temperature, weight, and signs of illness (not shown) showing that B15Rwt is representative of the WR virus stock. At higher virus doses (10^5–10^7 pfu per mouse), the difference in body temperature between viruses with or without the vIL-1βR was about 1°C 2–4 dpi, and hypothermia occurred earlier, between 3–5 dpi, probably due to a more rapid dissemination of VV (not shown).

Binding of Mouse IL-1α and IL-1β to vIL-1βR. The vIL-1βR binds human IL-1β with high affinity (K_D 234 pM), but not IL-1α (11), and binding to mouse IL-1β had been observed but the affinity was not reported (11, 12). Fig. 2*A* shows that similar doses of human and mouse IL-1β competitively inhibited the binding of human ^{125}I-IL-1β to the WR vIL-1βR to the same extent, indicating the vIL-1βR has similar affinity for each IL-1β. With cowpox virus, binding of human ^{125}I-IL-1β to the soluble receptor was inhibited slightly more efficiently with mouse than with human unlabeled IL-1β, suggesting a slightly higher affinity for the mouse cytokine (Fig. 2*A*). The specificity of vIL-1βR for mouse IL-1β and not IL-1α was observed with supernatants from VV WR (Fig. 2*B*), Tian-Tan, Lister, Wyeth, IHD-J, and IHD-W, and cowpox (not shown).

Expression of Soluble IL-1βRs in Infected Mice. IL-1βRs were detected after infection with 10^7 pfu of vB15Rwt but not in uninfected or vΔB15R-infected mice (Fig. 3*A*). These systemic soluble IL-1βRs very likely represent vIL-1βRs and not mouse soluble IL-1βRs because no activity was found in vΔB15R-infected mice. All of the samples containing IL-1βRs from brain, spleen, and lungs, but not plasma, had high virus titers (not shown). Infection with lower doses of WR (10^5 pfu per mouse) suppressed fever 1–5 dpi, despite the IL-1β binding activity in the brain being detected only 7 dpi, coinciding with high virus titers in this organ (not shown). Attempts to detect increased levels of IL-1β in plasma after infection with 10^7 pfu of vB15Rwt or vΔB15R using an ELISA with a detection limit of 10 pg/ml were unsuccessful (not shown).

vIL-1βR and Virus Replication in Mice. The production of soluble IL-1βRs might alter virus replication *in vivo*. However, no differences in virus titers were observed in lungs, spleen, and brain of mice intranasally infected with 10^7 pfu of vB15Rwt, vΔB15R, and vB15Rrev (Fig. 3*B*). Similar results were obtained in different experiments using various virus doses ranging from 10^3 to 10^7 pfu per mouse (not shown).

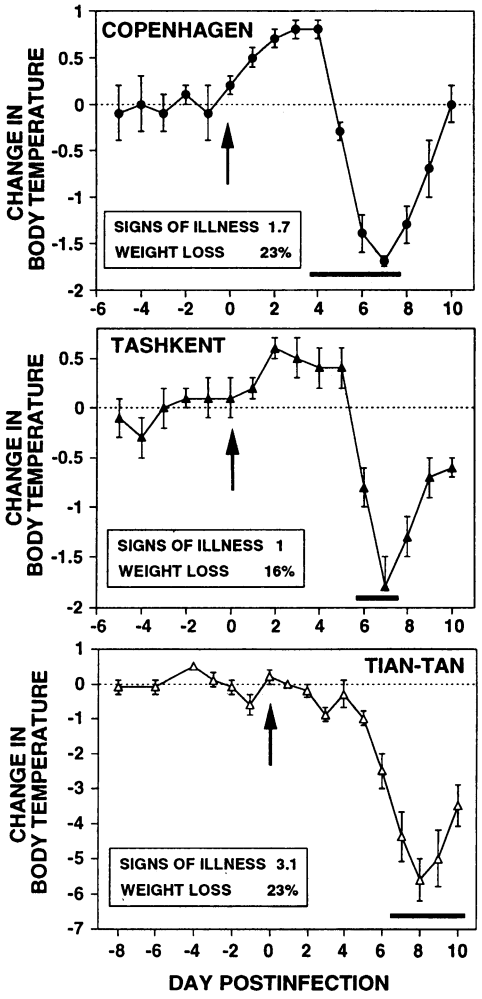

FIG. 4. Infection of mice with VV strains Copenhagen, Tashkent, and Tian-Tan. Groups of 10 mice were intranasally infected with VV Copenhagen (●; 5 × 10^7 pfu per mouse), Tashkent (▲; 10^6 pfu per mouse), or Tian-Tan (△; 10^6 pfu per mouse). The mean change in body temperature ± SEM and the basal temperature (broken line), which ranged from 35.9 to 36.3°C, are shown. Arrow indicates the day of virus inoculation. The horizontal bars indicate those days in which the signs of illness (scored from 0 to 4) and weight loss reached maximum levels, and the mean values for those days are shown in the Insets. No deaths occurred. Infection with other doses of Copenhagen (10^5, 10^6, or 10^7 pfu per mouse), Tashkent (10^5 pfu per mouse), and Tian-Tan (10^5 pfu per mouse) gave similar results (not shown).

Fever Is Induced in Mice Infected with Smallpox Vaccines Lacking the vIL-1βR. The febrile response after infection with smallpox vaccine strains, which do or do not express the vIL-1βR (11), was also investigated. Fig. 4 shows that mice infected with strains lacking the vIL-1βR, Copenhagen, and Tashkent, developed fever. In contrast, animals infected with Tian-Tan, which produces a receptor that binds mouse IL-1β well but human IL-1β poorly (11), failed to induce fever. All three strains induced hypothermia 6 dpi, which coincided with maximum signs of illness and weight loss (Fig. 4).

Hypothermia Correlates with Spread of VV to the Brain. Mice intranasally inoculated with 10^5 pfu of WR, Tian-Tan, and Copenhagen suffered different degrees of hypothermia, which correlated with the severity of infection. Hypothermia coincided with virus dissemination and its extent correlated with the virus yields recovered from the brain, but not from spleen or lungs. Mean temperatures of 6, 3, and 1°C below normal correlated with virus titers in the brain of 10^6, 10^4, and 10^3 pfu/g, respectively (not shown).

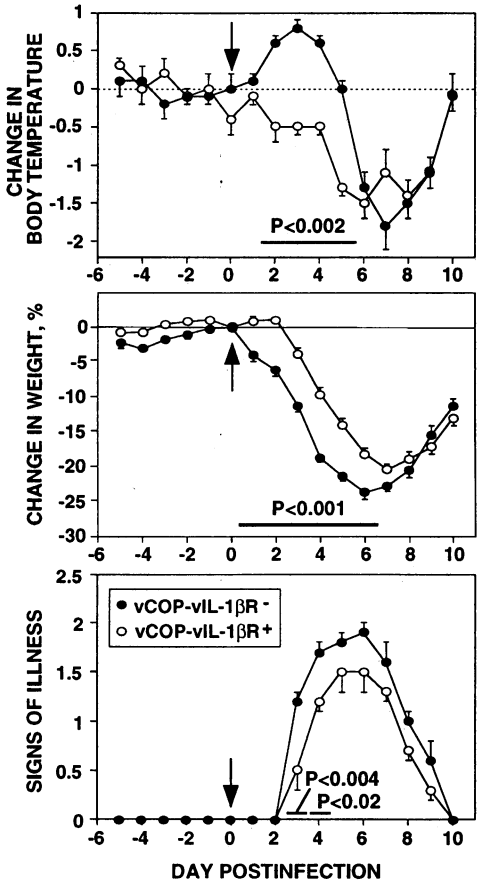

FIG. 5. Infection of mice with vCOP-IL-1βR⁻ and vCOP-IL-1βR⁺. Groups of 10 mice were intranasally infected with 10^7 pfu of vCOP-IL-1βR⁻ (●) or vCOP-IL-1βR⁺ (○). The mean change in body temperature ± SEM and the basal temperature (broken line), 36.1 and 36.2°C for vCOP-IL-1βR⁻ and vCOP-IL-1βR⁺, respectively, are shown. The mean change in weight ± SEM is expressed as the percentage of the mean weight of the group immediately prior to infection (solid line). Signs of illness were scored from 0 to 4 and the mean value ± SEM of each group is shown. No deaths occurred. Arrow indicates the day of virus inoculation. Horizontal bars indicate those days on which the difference between vCOP-IL-1βR⁻ and vCOP-IL-1βR⁺ was statistically significant when analyzed by Student's *t* tests, and the maximum *P* values are indicated. Similar results were observed at a virus dose of 5 × 10^7 pfu per mouse (not shown).

Expression of the vIL-1βR in Copenhagen Prevents Fever and Attenuates the Infection. To strengthen further the correlation between the vIL-1βR expression and suppression of fever, we investigated whether the expression of the vIL-1βR in VV Copenhagen would suppress the fever naturally induced by this virus. The vIL-1βR gene in the Copenhagen strain, named B16R (22), is inactivated by a nonsense mutation at codon 31. The WR B15R gene was inserted into the Copenhagen genome by transient dominant selection. Two viruses were isolated, which contained either the mutated B16R gene (vCOP-vIL-1βR⁻), representing a plaque-purified wild-type Copenhagen virus, or the active WR B15R gene (vCOP-vIL-1βR⁺). The expression of vIL-1βRs by these viruses was as predicted (not shown). Fig. 5 shows that infection with vCOP-IL-1βR⁺ prevented fever but vCOP-IL-1βR⁻-infected mice developed fever 2–4 dpi. In addition, expression of the vIL-1βR delayed the weight loss and reduced the signs of illness, similar to the effects produced in the WR strain.

Blockade of VV-Induced Fever with Antibodies to IL-1β. The induction of fever by IL-1β was tested further using an anti-IL-1β antibody. Microchip battery-free transponders implanted in the subcutaneous tissue of mice were used in these

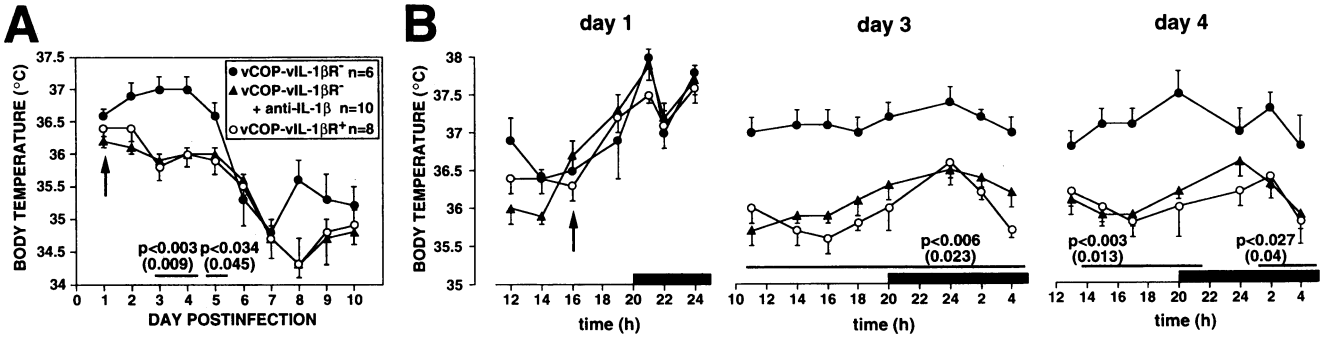

FIG. 6. Inhibition of fever in VV-infected mice with antibodies to IL-1β. Groups of mice were subcutaneously implanted with transponders and intranasally infected with 10^7 pfu of vCOP-IL-1βR⁻ (●, ▲) or vCOP-IL-1βR⁺ (○). After 24 h, mice were intraperitoneally injected with 200 μg of purified monoclonal antibody B112 to mouse IL-1β in 250 μl (▲) or the same volume of endotoxin-free phosphate buffered saline (●, ○). No deaths occurred. Arrow indicates the time of antibody inoculation. Horizontal closed boxes represent the dark period. Horizontal lines indicate those time points at which the difference between vCOP-IL-1βR⁻ and both vCOP-IL-1βR⁺ and vCOP-IL-1βR⁻ plus antibody were statistically significant when analyzed by Student's *t* tests, and the mean and maximum (in parentheses) *P* values are shown. (*A*) Mean body temperature ± SEM calculated from three determinations during the light period. (*B*) Mean body temperature ± SEM at different times during the indicated dpi.

experiments. In a preliminary experiment similar to that shown in Fig. 5, the difference between the mean body temperature in groups of 10 mice determined with transponders and their mean rectal temperature was 0.2 ± 0.01°C in 26 different comparisons (not shown). The body temperature during the light period was different in mice infected with vCOP-IL-1βR⁻ or vCOP-vIL-1βR⁺ (Fig. 6*A*), corroborating our previous findings (Fig. 5). Intraperitoneal inoculation of a neutralizing monoclonal antibody to mouse IL-1β 1 dpi blocked the fever normally induced by vCOP-IL-1βR⁻, and produced body temperatures indistinguishable from those of vCOP-vIL-1βR⁺-infected mice. Body temperature at 1 dpi followed a light/dark cycle that paralleled the activity of mice (Fig. 6*B*), similar to uninfected mice (not shown) and consistent with other studies (21, 23). In contrast, 3 and 4 dpi mice infected with vCOP-IL-1βR⁻ showed higher temperatures during both light and dark periods than those receiving the antibody to IL-1β or infected with vCOP-IL-1βR⁺. Inoculation of anti-IL-1β antibodies did not affect the body temperature in vCOP-IL-1βR⁺-infected mice, and delayed the weight loss and reduced the signs of illness of vCOP-IL-1βR⁻-infected mice, similar to the expression of the vIL-1βR in vCOP-IL-1βR⁺ (not shown).

DISCUSSION

We show here that VV prevents fever in infected mice by expressing a soluble IL-1βR. To our knowledge, this is the first description of a virus that inhibits fever in response to infection and the mechanism by which this inhibition operates. This finding provides direct evidence that IL-1β induces fever naturally during a virus infection and, in this model, is the principal endogenous pyrogen.

Inhibition of fever by VV correlates with soluble IL-1βRs in tissues and plasma in infected mice, and free IL-1β would consequently be neutralized. Importantly, fever was efficiently inhibited when neither virus replication nor soluble IL-1βRs were detected in the brain, suggesting that the vIL-1βR neutralizes circulating IL-1β. This was confirmed by the inhibition of fever with peripheral injection of antibodies to IL-1β. The failure to detect increased levels of IL-1β in plasma in vΔB15R-infected mice may reflect low levels of free circulating IL-1β during fever or a technical difficulty with immunoassays (1, 24).

Poxviruses express soluble cytokine receptors that could inhibit other putative endogenous pyrogens. The vIL-1βR does not bind IL-1α or other pyrogenic cytokines such as IL-6, TNF-α, IFN-α/β, or IFN-γ, and the VV strains used here do not express soluble receptors for IL-6 or TNF-α (refs. 11, 14, and 19; this report; unpublished data). Although VV encodes receptors for IFN-γ and IFN-α/β, the former does not inhibit mouse IFN-γ (14, 19, 25, 26). All of these cytokines are induced by poxvirus infections in cultured spleen cells and animal models (27–30), and their failure to induce fever when IL-1β is inhibited suggests that IL-1β is the major endogenous pyrogen in poxvirus infections. A minor contribution of IFN-α/β to fever in infections with VV lacking IFN-α/β receptors remains possible, although IL-1β is sufficient to induce fever.

Variola virus caused smallpox, a systemic poxvirus infection associated with high fever (6). Interestingly, the vIL-1βR gene is inactive in variola virus (31–33). This observation correlates with the induction of fever in mice infected with VVs that lack the vIL-1βR. Variola virus does, however, have genes predicted to encode receptors for IFN-α/β, IFN-γ, and TNF-α/β suggesting that fever during smallpox was not mediated by these cytokines, consistent with a central role of IL-1β in poxvirus-induced fever.

The VV B13R protein is homologous to cowpox *crmA*, which inhibits the IL-1β converting enzyme (34), and may reduce IL-1β production. However, as the vIL-1βR determines the onset of fever in strains WR and Copenhagen, which do or do not express B13R, respectively (35), it is unlikely that B13R plays a major role modulating IL-1β systemic effects such as fever.

The inhibition of fever by the vIL-1βR and antibodies to IL-1β is consistent with IL-1β being the major soluble form of IL-1 and the mediator of systemic effects (13). Consistent with this, IL-1β-deficient mice are resistant to turpentine-induced fever, which also demonstrates a critical role for IL-1β in fever (23). The complete blockade of VV-induced fever by antibodies to IL-1β is remarkable since anti-IL-1β antibodies failed to completely block lipopolysaccharide-induced fever and IL-1β-deficient mice are only partially resistant to lipopolysaccharide-mediated fever (1, 21, 36). IL-6 produced in the central nervous system, but not in the periphery, has been shown to act downstream of IL-1β and lipopolysaccharide in the induction of fever (20); thus, blockade of central IL-6 might also suppress VV-induced fever. Other putative endogenous pyrogens may play a role with other pathogens. For example, antibodies against TNF-α prevent fever in malaria (37).

Although few reports compared smallpox vaccination with different vaccine strains in the same population (6) some observations are consistent with our results. Vaccination with strain Copenhagen (vIL-1βR⁻) produced a higher and more prolonged morbidity and fever than strain Lister (vIL-1βR⁺), and strain Tashkent (vIL-1βR⁻) produced a strong reaction that caused its replacement by safer vaccines (6). Although strain Lister (vIL-1βR⁺) still induced fever, this was influenced by the site of inoculation (6). During local virus repli-

cation after vaccination, the vIL-1βR may not be synthesized in sufficient quantities to neutralize systemic IL-1β and may determine the degree rather than the absolute appearance of fever. In contrast, as shown here, in systemic infections the vIL-1βR efficiently blocks the febrile response.

The severe hypothermia in mice infected with VV, which did not occur in VV or variola infections of humans (6), is unrelated to vIL-1βR expression, but attributable to systemic infection and in particular replication in the brain, where the virus replicates in the meninges but not neurons (38, 39). Infection of mice with other pathogens has induced hypothermia (40–42).

Beneficial effects of fever (1, 2) such as increased host survival were not observed here, but might be apparent in other models of poxvirus infection, such as myxoma and ectromelia viruses in their natural hosts where virus virulence increases at lower body temperatures (43, 44). Replication of vΔB15R was also not restricted by fever and this virus reached similar titers compared with wild-type and revertant viruses in lungs, spleen, and brain. This contrasts with the reported attenuation of a B15R knockout virus after intracranial inoculation of mice (12). Our construction and analysis of the revertant virus confirmed that the enhanced virulence of the vΔB15R we observed previously (11) is attributable wholly to the loss of vIL-1βR.

The vIL-1βR is expressed by attenuated smallpox vaccine strains (11). Consistent with this, expression of the vIL-1βR in the smallpox vaccine Copenhagen, which produced high frequencies of postvaccinial complications (6), reduced the severity of the disease. Expression of cytokines from VV has been proposed to increase the safety of recombinant vaccines (45, 46). Alternatively, expression of virus-encoded immune modulators such as the vIL-1βR may reduce the overreaction of the host immune response to vaccination, which is believed to have been responsible for some of the complications after smallpox vaccination (6).

This study illustrates the use of VV to investigate the physiological roles of cytokines. Experimental inoculation of cytokines induces a transient elevation of body temperature but does not demonstrate a role in naturally occurring fever (1). VV induces physiological doses of cytokines that produce fever and, simultaneously, synthesizes cytokine inhibitors that are delivered at the site of infection and can reach systemic levels. This experimental approach circumvents the need to demonstrate a correlation between fever and increased cytokine levels, which may be undetectable or may not reach circulation.

In conclusion, we show that VV suppresses the febrile response in the infected host by blocking the activity of IL-1β with a specific soluble receptor. This shows that IL-1β is the principal endogenous pyrogen in a poxvirus infection. These studies show that analysis of the mechanisms that viruses use to modulate the host immune response can increase our knowledge of not only virus pathogenesis but also the immune system.

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