Type I IFN Modulates Innate and Specific Antiviral Immunity


*J Immunol* 2000; 164:4220-4228; doi: 10.4049/jimmunol.164.8.4220

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Type I IFN Modulates Innate and Specific Antiviral Immunity

Joan E. Durbin,*§ Ana Fernandez-Sesma,† Chien-Kuo Lee,* T. Dharma Rao,‡ Alan B. Frey,§ Thomas M. Moran,† Stanislav Vukmanovic,§ Adolfo García-Sastre,† and David E. Levy*‡§

IFNs protect from virus infection by inducing an antiviral state and by modulating the immune response. Using mice deficient in multiple aspects of IFN signaling, we found that type I and type II IFN play distinct although complementing roles in the resolution of influenza viral disease. Both types of IFN influenced the profile of cytokines produced by T lymphocytes, with a significant bias toward Th2 differentiation occurring in the absence of responsiveness to either IFN. However, although a Th1 bias produced through inhibition of Th2 differentiation by IFN-γ was not required to resolve infection, loss of type I IFN responsiveness led to exacerbated disease pathology characterized by granulocytic pulmonary inflammatory infiltrates. Responsiveness to type I IFN did not influence the generation of virus-specific cytotoxic lymphocytes or the rate of viral clearance, but induction of IL-10 and IL-15 in infected lungs through a type I IFN-dependent pathway correlated with a protective response to virus. Combined loss of both IFN pathways led to a severely polarized proinflammatory immune response and exacerbated disease. These results reveal an unexpected role for type I IFN in coordinating the host response to viral infection and controlling inflammation in the absence of a direct effect on virus replication. The Journal of Immunology, 2000, 164: 4220–4228.

Interferons were discovered on the basis of their antiviral activity against influenza virus (1). Two distinct families of IFNs (type I or α/β and type II or γ) can be distinguished on the basis of primary sequence homology and use of distinct cell surface receptors; however, their signaling mechanisms partially overlap, leading to the activation of a partially overlapping set of genes (reviewed in Refs. 2–4). Both types of IFN activate intracellular protein tyrosine kinases of the Jak family, leading to the phosphorylation and activation of Stat transcription factors. The Stat1 protein is required for signaling from both type I and type II IFN (5). In fact, ablation of the Stat1 gene through gene targeting produced animals exhibiting total resistance to the action of IFN, rendering them highly susceptible to viral and microbial pathogens (6, 7). In addition to their antiviral action, a variety of immunomodulatory and antiproliferative activities have been ascribed to IFNs. The biological relevance of these nonantiviral effects has remained elusive, particularly in the case of type I IFN (8).

The direct antiviral effects of IFN are mediated by induction of a set of IFN-stimulated genes. The precise mechanisms of action of these genes in “interfering” with viral replication are not well understood, and viruses differ in their sensitivity to IFN (9). We have previously demonstrated (10) that mice lacking Stat1 consistently show increased sensitivity to infection with the influenza virus strain A/PR/8/34 (PR8). Surprisingly, the susceptibility of Stat1−/− animals to this pathogen did not reflect a defect in innate antiviral immunity. In contrast to the response to other pathogens (6, 7), Stat1−/− mice were capable of preventing uncontrolled viral replication and of clearing PR8 virus. PR8 virus titers and the kinetics of clearance in Stat1−/− mice were comparable with those seen in wild-type animals (10). Nonetheless, PR8 virus infection produced enhanced lethality in Stat1−/− mice.

It has been demonstrated that the pathology resulting from influenza virus infection results substantially from host inflammatory processes rather than directly from virus-mediated damage to respiratory epithelium (11), particularly in the absence of the Mx gene product, which is defective in most inbred strains of mice (9). We took advantage of this model to explore the role of IFNs in the host response and development of Ag-specific immunity to influenza virus infection by comparing the course of a primary PR8 virus infection in the absence of responsiveness to type I IFN, type II IFN, or both. We found that in the absence of Stat1, influenza virus infection produces a distinctive, proinflammatory pathological process. This exacerbated disease correlates with a lack of the normal, strong induction of IL-15 by influenza virus infection and with a Th2-biased host immune response. Although a Th2-type cytokine profile was present in infected lungs of IFN-γ−/− animals, this polarized response appeared to be largely compensated by the effects of type I IFN. The converse was not true; in the absence of the IFN-α receptor, IFN-γ produced in response to virus infection was not sufficient to prevent enhanced disease.

Materials and Methods

Mice

The production of Stat1−/− mice on a CD1 background has been previously described (6). For comparison with mice disrupted for the IFN-γ gene on the C57BL6 background and with IFN-α receptor knockout or IFN-α-γ receptor double knockout mice on the 129Sv/Ev background, Stat1−/− mice were backcrossed to C57BL6 or to 129Sv, respectively (8th backcross generation). Similar results to those described here were also observed with Stat1−/− mice carrying a distinct mutation (7) that were maintained on a 129Sv/Ev background (data not shown). IFN-γ−/− mice (12) were purchased from The Jackson Laboratory, Bar Harbor, ME. Mice with targeted mutations at the IFN-α receptor locus (IFNAR−/−) and at both the IFN-α receptor and IFN-γ receptor loci (IFNAR/GR−/−) (13, 14) were kindly provided by Michel Aguet (Lausanne Switzerland) and Robert

Address correspondence and reprint requests to Dr. David E. Levy, Department of Pathology, New York University School of Medicine, 550 First Avenue, New York, NY 10016. E-mail address: levyd01@med.nyu.edu

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Preparation of bone marrow-derived macrophages

Marrow harvested from four femurs of each mouse strain were plated in a six-well tissue culture dish in DMEM supplemented with 10% mouse L cell-conditioned medium and 20% FCS (Life Technologies). The medium was changed after 48 h, and adherent cells were allowed to grow to near confluence. For FACS analysis, cells were dislodged by scraping and then stained with FITC-conjugated hamster anti-mouse CD11b (clone 500-A2, Caltag). Individual wells were left untreated, treated with LPS at 5 μg/ml (from Escherichia coli strain 0127:B8, Sigma, St. Louis, MO), or infected with 10⁵ PFU influenza virus A/WSN/33. After 24 h incubation, cells were lysed in TRIZOL and RNA extracted with phenol-chloroform.

Cellular cytotoxicity assay

CTL activity from mice that had survived infection with influenza virus was measured on autologous cells. Spleen cells from virus-infected mice were treated to lyse RBC and divided into three samples. One sample was irradiated for use as stimulator cells by infecting with influenza A/PR/8/34 virus at a multiplicity of infection of 4. Stimulators were incubated together with a second sample of spleen cells (responders) at a ratio of 4 × 10⁵ stimulators plus 4 × 10⁶ responders/ml for 5 days at 37°C. A third sample of splenocytes was stimulated with 25 μg/ml Salmonella typhosa LPS for 5 days at 2 × 10⁶ cells/ml and used as targets in cytotoxicity assays. Virus-specific cytotoxicity was measured by mixing spleen cells at a ratio of 40:1 with autologous target cells labeled with sodium ¹¹¹Ijchromate, either uninfected or infected with influenza PR8 virus at a multiplicity of infection of 10. Cells were incubated for 4 h at 37°C and pelleted, and radioactive release into supernatants was measured. Maximal release was measured by incubating target cells with 0.5% Nonidet P-40 and spontaneous release by incubating target cells with medium alone (routinely <10% of maximal release). Percent cytotoxicity was calculated as [(test release – spontaneous release)/(maximal release – spontaneous release)] × 100. Ab-directed lysis, a measure of activated CD8 T lymphocytes, was determined by similar assays but using ¹¹¹Ij-labeled P815 tumor cells coated with anti-CD3 Ab 2C11 as targets as previously described (15).

Results

Influenza A/PR/8/34 virus clearance was unimpaired in Stat−/− mice

We have previously demonstrated an increased sensitivity of Stat−/− mice relative to wild-type animals to infection by human influenza virus strain A/PR/8/34 (10). Stat−/− mice infected i.n. with this virus displayed an LD₅₀ 10-fold lower than their wild-type counterparts. This increased sensitivity could not be explained by increased virus replication in the absence of IFN responsiveness, because peak virus titers were comparable in lungs harvested from infected wild-type or mutant animals. When viral titers were determined throughout the course of the infection, the kinetics of viral clearance were comparable in wild-type, Stat−/−, and IFNAR−/− mice (10).

To characterize the cellular immune response to influenza virus, CTL activity was measured with the use of splenocytes derived from C57BL/6 mice immunized in vivo by i.p. injection of a sublethal dose of PR8 virus (Fig. 1). Both wild-type and Stat−/− mice generated cytotoxic lymphocytes capable of killing target cells pulsed with the immunodominant influenza virus peptide presented by H-2Db (16). Cytotoxicity measurements over a range of peptide concentrations or with virus-infected target cells showed no significant differences between wild-type and Stat−/− effectors.

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Additional information

1 Abbreviations used in this paper: HA, hemagglutination; i.n., intranasal(ly); TCID, tissue culture infectious dose.
bred onto each of the different strain backgrounds 129, C57BL6, and outbred CD1.

In wild-type animals, infected airways were surrounded by a small cuff of responding lymphocytes by day 6 post inoculation (Fig. 2, A and E). Inflammation in IFN-γ−/− mice was similar to wild-type, although somewhat more cellular (Fig. 2, C and F), consistent with previous reports showing little differential sensitivity to influenza virus infection in animals lacking IFN-γ (18). In contrast, lungs from infected Stat1−/− mice (Fig. 2, B and H) showed a markedly exacerbated inflammatory process. Infiltrates were much more cellular and diffuse. In addition to the increased number of inflammatory cells, the character of the infiltrates was changed in the Stat1−/− mice. Although small numbers of lymphocytes were identified in the infected tissue, the majority of responding cells were granulocytes, and infiltrating macrophages, neutrophils, and eosinophils were abundant (Fig. 2H). Interestingly, whereas the inflammatory process detected in lungs harvested from IFNAR−/− animals (Fig. 2D) was significantly worse than that in wild-type or IFN-γ−/− mice, the severity was consistently intermediate between that observed in wild-type relative to Stat1−/− animals. Similar to the Stat1−/− mice, the responding inflammatory cells in PR8 virus-infected IFNAR−/− mice were primarily (>50%) granulocytes (Fig. 2G). However, fewer infiltrating cells and less tissue destruction were observed relative to infected Stat1−/− tissue (compare Fig. 2B and Fig. 2D). The histopathology of PR8 virus-infected lungs from IFNAR/GR−/− mice was comparable with that seen in the Stat1−/− animals (data not shown), suggesting that both IFN-α and IFN-γ contributed to regulation of the inflammatory response to influenza virus infection.

Humoral immune response to influenza virus infection in Stat1-deficient and wild-type mice

Serum samples were taken from wild-type and Stat1−/− mice before and after i.n. inoculation with the attenuated influenza virus strain, NA/B-NS (17), and were assayed for the presence of virus-specific Abs. Inflammation-virus-specific Ig was detected at equivalent levels in animals of both genotypes (Fig. 3A). Interestingly, whereas wild-type animals produced Abs against influenza virus of both IgG1 and IgG2a subclasses, no IgG2a virus-specific Abs were detected in serum from infected Stat1−/− mice. Despite these differences in Ab isotype, both wild-type and Stat1−/− mice displaying detectable anti-influenza virus Abs were fully protected from rechallenge with a lethal dose of WSN virus (data not shown).

Heavy chain class switch to IgG2a is stimulated in response to IFN-γ (19), suggesting that the lack of this Ig isotype was the result of a direct loss of the ability of cells to respond transcriptionally to IFN-γ in the absence of Stat1. Preferential production of the IgG1 subclass of Abs is characteristic of B lymphocytes maturing under the influence of Th2 cells (20). A hallmark of Th2-influenced responses, which normally characterize allergic, anti-parasitic, or autoimmune responses rather than antiviral ones, is the production of IgE. We detected abnormally high levels of total IgE in the serum of influenza virus-infected Stat1−/− mice (Fig. 3B), and even naive animals showed significant levels of circulating IgE. IgE levels were below the limit of detection (<0.04 ng/ml) in control animals, whether or not they were infected with virus. Thus, Stat1−/− mice displayed a default Th2 bias that was greatly augmented by viral infection. Neither the enhanced production of IgE nor the complete suppression of IgG2a synthesis were noted in influenza virus-infected IFN-γ−/− mice (18), although a heavy bias toward IgG1 was noted previously in IFNAR/GR−/− mice.
These results strongly suggest that type I IFN in addition to type II IFN controls heavy chain class switch recombination.

*Th2 cytokine production in Stat1−/− mice*

T cell responses in the absence of IFN signaling were further characterized by comparison of wild-type and Stat1−/− splenic lymphocyte differentiation. Th1 and Th2 T cell subsets are defined functionally in terms of the cytokines that they produce (21). The high levels of IgE found even in serum from naive Stat1−/− mice suggested an intrinsic bias toward Th2-influenced responses. Therefore, we measured cytokines produced by spleen cell cultures isolated from uninfected wild-type and Stat1−/− mice (Table I). Splenocytes were stimulated in vitro through the TCR and allowed to differentiate for 1 wk in the presence of IL-2. Restimulation of Stat1−/+ splenocytes led to exclusive production of IFN-γ. In contrast, significant amounts of IL-5 were secreted by rested Stat1−/−

![Image](https://via.placeholder.com/150)

**FIGURE 2.** Distinct inflammatory response to influenza virus in Stat1−/− and IFNAR−/− mice. Photomicrographs show histological sections of lungs from wild-type 129 mice, Stat1−/− 129 mice, IFN-γ−/− C57BL6 mice, and IFNAR−/− 129 mice 6 days after i.n. infection with 100 TCID of influenza virus A/PR/8. A–D, ×20 views of infected lungs; E–H, ×100 magnifications to show infiltrating cells. A and E, Lungs from wild-type mice showing peribronchial and bronchiolar cuffs of lymphocytes. m, a smooth muscle cell of the airway. B and H, Diffuse inflammatory process involving the entire lung of the Stat1−/− animals with inflammatory cells consisting entirely of macrophages, neutrophils, and eosinophils infiltrating between the epithelial lining of an airway (ep) and its muscular coat (m). C and F, IFN-γ−/− lung inflammation is peribronchial and largely lymphocytic. D and G, IFNAR−/− mouse lung with patchy bronchopneumonia of mixed peribronchial and perivascular infiltrates consisting of lymphocytes and acute inflammatory cells. In addition to bronchitis, the infiltrating cells extended into lung parenchyma.

![Image](https://via.placeholder.com/150)

**FIGURE 3.** Enhanced Th2-type Ig production in Stat1−/− CD1 mice. A, Influenza virus-specific Ig levels were measured by ELISA. Data represent averages of sera from three wild-type (□) or Stat1−/− mice (■). B, Circulating IgE levels are increased in Stat1−/− mice. IgE levels in sera from naive (left) or influenza virus-infected mice (right) were measured by ELISA. The levels of IgG2a and IgE were significantly different between wild-type and Stat1−/− (p < 0.01).
expected IFN-γ splenocytes were found to make mRNA corresponding to the ex-
generated IFN-γ. By this method, wild-type controls. Moreover, re-
stimulation of Stat1−/− cells produced significant production of the Th2-type cytokines IL-4, IL-5, and IL-10 not seen in wild-type cultures, in addition to IFN-γ.
This bias toward Th2 cytokine secretion in the presence of an ongoing Th1-like IFN-γ-producing response was also observed when cytokine transcripts from in vitro differentiated splenocytes were assayed by RNase protection. By this method, wild-type splenocytes were found to make mRNA corresponding to the expected IFN-γ and IL-2, as well as IL-10 (Fig. 4A, lane 11). Stat1−/− splenocytes, differentiated and assayed in parallel, produced transcripts corresponding to IL-5, IL-9, IL-10, and IL-13 as well as comparable amounts of IL-2 and IFN-γ (Fig. 4A, lane 12). Cytokine mRNA profiles varied somewhat with strain background in these experiments. Lymphocytes harvested from wild-type 129 animals were much more Th0 in character than those from C57BL6 or CD1 mice after in vitro differentiation and restimulation with anti-CD3 and anti-CD28. Nonetheless, both Stat1−/− (Fig. 4A) and IFNAR−/− (data not shown) splenocytes showed a marked Th2 bias compared with wild-type, strain matched controls.
The production of Th2-type cytokines by stimulated Stat1−/− splenocytes in the presence of ongoing production of Th1-type cytokines such as IFN-γ suggested a mixed response, possibly due to the absence of IFN-γ-mediated inhibition. Because unfractionated splenocytes were used for the in vitro differentiation and restimulation, the production of IFN-γ could reflect contributions from cell types other than CD4 helper cells, such as CD8 or NK cells. To dissect the contribution of CD4 and CD8 cells to these cytokine profiles, we measured cytokine protein levels on a per cell

Table I. Stat1−/− splenocytes are biased toward a Th2 phenotype

<table>
<thead>
<tr>
<th>Anti-CD3</th>
<th>IFN-γ (ng/ml)</th>
<th>IL-4 (ng/ml)</th>
<th>IL-5 (ng/ml)</th>
<th>IL-10 (ng/ml)</th>
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<td>Stat1−/−</td>
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<td>50.9</td>
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<td>&lt;0.2</td>
</tr>
<tr>
<td>Stat1−/−</td>
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<td>25.8</td>
<td>&lt;0.2</td>
<td>2.3b</td>
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</tbody>
</table>

* Spleen cell cultures were stimulated in vitro with anti-CD3 Ab, allowed to differentiate in culture for 1 wk, and then either left untreated (−) or restimulated with anti-CD3 (+), and cytokine levels were assayed by ELISA.

a Significantly different from wild type (p < 0.001).
b Significantly different from wild type (p < 0.01).

![FIGURE 4](http://www.jimmunol.org/DownloadedFrom) Distinctive lung cytokine profiles are induced by influenza virus infection in wild-type, Stat1−/−, IFN-γ−/−, and IFNAR−/− mice as determined by RNase protection assays. A. Lung RNA samples from five wild-type (lanes 1–5) and 5 Stat1−/− (lanes 6–10) CD1 animals 3 d after i.n. inoculation with PR8 virus. In vitro differentiation of splenocytes gave rise to Th1-type cytokine profiles in wild-type (lane 11) and Th2-type cytokine profiles from Stat1−/− cells (lane 12). B. Lung RNA from 3 wild-type (lanes 1–3) and 3 Stat1−/− (lanes 4–6) mice 6 days after i.n. inoculation with PR8 virus. C. Twelve IFN-γ−/− C57BL6 mice were inoculated with PR8 virus. Samples for RNase protection assay were harvested 3 days (lanes 1–4), 6 days (lanes 5–8), and 9 days (lanes 9–12) after i.n. inoculation. D. Lung RNA samples from four wild-type 129 mice 3 days after PR8 virus infection (lanes 1–4). RNA from lungs of PR8 virus-infected IFNAR−/− 129 mice harvested 3 days (lanes 5–7) and 6 days (lanes 8–10) postinfection. All infections were i.n. using 100 TCID of PR8 virus. RNase protection assays used 25 μg RNA samples and the PharMingen mCK-1 multiprobe template set. Unprotected probe is used as a size marker for each assay (M). E. Lymphocytes producing IFN-γ and IL-5 were scored by intracellular cytokine staining and for cell surface CD4 or CD8 phenotype by flow cytometry. The percentages of CD8 cells (upper) or CD4 cells (lower) producing IL-5 (upper left quadrants) or IFN-γ (lower right quadrants) are indicated.
basis by flow cytometry (Fig. 4E). We scored IFN-γ-producing cells as a marker for Th1-type and IL-5-producing cells as a marker of Th2-type. Approximately one-half of the differentiated wild-type CD4 cells produced IFN-γ and very few produced IL-5, indicative of a Th1 response. In contrast, few Stat1−/− CD4 cells produced IFN-γ, whereas nearly 10% produced IL-5. The majority of CD8 cells of both genotypes produced IFN-γ. Therefore, the majority of Stat1−/− CD4 cells would appear to be biased toward a Th2 response or at least against a Th1 response, and the vast majority of T lymphocyte-derived IFN-γ produced by Stat1−/− cultures was not from helper T cells but rather from CD8 cells.

Cytokine production in infected lung tissue

We examined whether this Th2 bias observed with in vitro differentiated Stat1−/− splenocytes was also present at the site of infection. Fig. 4, A–D, shows cytokine mRNA profiles from lungs of wild-type, Stat1−/−, IFN-γ−/−, and IFNAR−/− mice, harvested at various times after i.n. inoculation with PR8 virus. On day 3 of infection, lungs from wild-type animals (Fig. 4A, lanes 1–6) showed strong induction of IL-6 and IL-15, and a clear but less robust induction of IFN-γ. By day 6 (Fig. 4B), the IL-15 signal had begun to wane, and a delayed IL-10 induction was evident. This pattern of cytokine gene induction, consistently present in each animal assayed, was distinct from that seen in lungs from PR8 virus-infected Stat1−/− mice. In Stat1 mutant animals, IL-6 and IFN-γ were also induced strongly by day 3 (Fig. 4A, lanes 6–10), similar to wild-type animals. However, missing from the Stat1−/− profile at day 3 was the strong induction of IL-15 transcripts, but present was weak induction of the Th2 cytokines IL-5 and occasionally IL-13. Missing from the 6-day infected Stat1−/− lungs was the induction of IL-10 seen in wild-type samples (Fig. 4B). This pattern of cytokine expression within the infected tissue was quite different from that seen in differentiated splenocytes and could not be classified in terms of a simple Th1-Th2 dichotomy. Therefore, although the presence of Th2 cytokines may have contributed to disease exacerbation in Stat1−/− animals, it is likely that Th2 cytokines alone were not sufficient and additional factors influenced the altered pathogenesis observed.

This conclusion was reinforced by examination of the cytokine response in virus-infected IFN-γ−/− mice (Fig. 4C). Animals were infected i.n. with PR8 virus, and groups of four were sacrificed at 3-day intervals and analyzed individually. Lung samples harvested at day 3 showed strong induction of IL-6 and IL-15 (Fig. 4C, lanes 1–4). Similar to wild-type animals, IL-10 was induced by day 6. Unlike wild-type animals, however, IL-5 and IL-13 were also occasionally detected in lungs of animals harvested on day 6 (lanes 5 and 8) similar to the pattern observed in Stat1−/− samples and indicative of the Th2 bias observed in the absence of IFN-γ. This result suggests that the presence of IL-5 and IL-13 were not sufficient to cause significant disease exacerbation, at least in the presence of an intact type I IFN response, and reinforces the conclusion that the mere presence of Th2 cytokines is insufficient to explain the altered pathology of virus-infected Stat1−/− mice.

Fig. 4D shows results of influenza virus infection in IFNAR−/− mice. In PR8 virus-infected lungs harvested on day 3, IL-6 and IFN-γ transcripts were detected in all animals (Fig. 4D, lanes 1–5). Juxtaposition of samples from wild-type, background-matched controls (Fig. 4D, lanes 1–5) underscores the reduction in IL-15 induction in the absence of IFN-α responsiveness (Fig. 4D, lanes 6–10). No increase in IL-5 or IL-13 was detected by RNase protection assay in these animals. IL-10 induction was present in the day 6 IFNAR−/− lung samples, consistent with kinetics seen in wild-type animals. The increased IFN-γ mRNA synthesis seen in the IFNAR−/− mice from 3 to 6 days (Fig. 4D, lanes 6–10) was consistent between experiments and not seen with other mutant strains.

Macrophage cytokine production in vitro

We examined potential sources of IL-15 mRNA detected in influenza virus-infected lungs of wild-type and IFN-γ−/− mice. Macrophages were derived from each mouse strain by culturing bone marrow for 7 days in L cell-conditioned media. Homogeneity of cultured cells was estimated to be between 80 and 90% by FACS analysis after staining for CD11b (data not shown). Monolayers of adherent macrophages were treated with LPS (5 μg/ml) or infected at high multiplicity with influenza virus A/WSN/33. RNA was isolated from each culture after 24 h and cytokine transcripts were analyzed by RNase protection. As expected (22, 23), IL-15 was strongly induced by LPS in wild-type macrophages derived from 129SvEv or C57BL6 mice (Fig. 5, left). Cytokine profiles from IFN-γ−/− cells resembled wild-type controls (Fig. 5A, right). However, no IL-15 induction occurred in LPS-treated IFNAR−/− or Stat1−/− macrophages. IL-15 was also induced in wild-type and IFN-γ−/− cultures in response to virus infection, although to a lesser extent than by LPS. It is likely that type I IFN induction by either stimulus mediates the increased transcription of IL-15. IL-10 induction paralleled that of IL-15 in response to LPS or virus, whereas IL-6 was induced in all strains regardless of genotype only in response to LPS. Thus, the prominent induction of IL-15 observed in infected lungs likely originated from macrophages.

FIGURE 5. Macrophage cytokine mRNA profiles. Bone marrow-derived macrophages were cultured from wild-type, Stat1−/−, and IFN-γ−/− C57BL6 mice (A) as well as wild-type, Stat1−/−, and IFNAR−/− 129 mice (B). Macrophages of each strain/genotype were left untreated or treated for 24 h with LPS (5 μg/ml) or 105 PFU influenza virus A/WSN/33, as indicated. IL-15, IL-10, and IL-6 transcripts were strongly induced by LPS in wild-type and IFN-γ−/− macrophages. Virus produced a more modest induction of IL-15 and IL-10. Only IL-6 production was induced in IFNAR or Stat1−/− macrophages.
either resident or infiltrating. IL-10, found in infected lungs of all but Stat1−/− mice, may have been elaborated by other cell types in addition to macrophages (24, 25) since IFNAR−/− macrophages appear deficient in IL-10 production, and yet this cytokine was clearly induced in infected lungs (see Fig. 4D).

Discussion

Both type I and type II IFN are known to be involved in important aspects of the host defense against infectious disease. Type I IFN has been largely characterized for inducing a cell-autonomous antiviral state and type II IFN for modulating the immune response. Using mice lacking the Stat1 transcription factor, and therefore unable to respond transcriptionally to either type of IFN, we have examined the host response to influenza virus infection. Influenza viral disease is largely caused by the host response rather than by direct cytopathology. Use of influenza A/PR/8 strain bypassed the differential permissivity for virus replication that was observed with other viruses in the absence of IFN responses (6, 7, 10) and allowed direct examination of the host immune response. PR8 virus titers rose and fell in wild-type and mutant animals to similar levels and with equal kinetics (10) and yet produced exacerbated disease in Stat1−/− mice, allowing us to study the role of both type I and type II IFN in the development of a protective, virus-specific immune response.

We have observed a markedly different disease process in influenza virus-infected Stat1−/− mice that is characterized by a proinflammatory response mediated by diffuse, largely granulocytic pulmonary infiltrates. Stat1−/− mice were ~10-fold more susceptible to lethal infection than congenic wild-type, IFN-γ−/−, or IFNAR−/− strains, although the virulence of the PR8 virus strain makes subtle alterations in sensitivity difficult to measure by LD50. However, comparative histology of lungs harvested from infected animals reinforced this conclusion, consistently showing a gradation of pathology, from primarily lymphocytic bronchitis in wild-type and IFN-γ−/− animals, to patchy bronchopneumonia with mixed inflammatory infiltrates in IFNAR−/− mice, to diffuse, severe bronchopneumonia in Stat1−/− animals. The inflammation differed not only in terms of amount but also by its composition. Although lymphocytes were clearly recruited to the site of infection in all infected animals, there was a predominance of macrophages and acute inflammatory cells in lungs of type I IFN-nonresponsive animals not seen in wild-type mice. Inflammation in the IFN-γ−/− mice was largely lymphocytic, although it was somewhat more mixed than in wild-type mice. In contrast, the proportion of granulocytes increased in the absence of the type I IFN receptor (50–60%) and became overwhelming in the absence of Stat1.

It is likely that some component of the distinct histopathology and survival between influenza virus-infected wild-type and Stat1 mice can be explained by differences in helper T cell subsets responding to infection. An inappropriate Th2 bias was observed in Stat1−/− mice both in vitro and in vivo. The resulting production of IL-5 and IL-13 at the site of infection may explain some part of the deviation from a typical antiviral Th1 response as well as the proinflammatory nature of the response. Graham et al. (26) have demonstrated the requirement for Th1 cells in adoptive transfer experiments using influenza virus-specific Th1 or Th2 CD4+ cell clones. Th1 clones provided protection against lethal challenge whereas transfer of Th2 clones was nonprotective and led instead to disease exacerbation. However, the Th2-biased cytokine profile in infected IFN-γ−/− mice was accompanied by a very different disease pathology and outcome than observed in the absence of type I IFN responsiveness, suggesting that Th2 cytokines alone cannot be the full explanation for the Stat1−/− phenotype.

IFN-γ−/− animals responded to influenza virus in a manner very similar to that of wild-type animals, as previously observed by others (18). Although IFN-γ−/− mice produced more virus-specific IgG1 than wild-type animals, they nonetheless displayed significant levels of heavy chain class switching to IgG2a and did not display increased concentrations of IgE (18). In contrast, Stat1−/− animals produced no antiviral IgG2a Abs and displayed high levels of circulating IgE. It is therefore likely that type I IFN- and Stat1-mediated events were of major importance in shaping the normal, protective, antiviral response that consisted of both IgG1 and IgG2a anti-influenza Abs as well as the absence of IgE. The absence of IFN-γ signaling alone, despite the presence of Th2 type cytokines in lungs of influenza virus-infected IFN-γ−/− mice, was not sufficient to increase disease severity or to bias the primary immune response to the degree observed in the Stat1−/− animals.

We have observed a strong induction of IL-15 mRNA in infected lungs of the relatively protected wild-type and IFN-γ−/− animals and reduction or absence of its induction in IFNAR−/− and Stat1−/− animals which showed exacerbated disease. IL-15 transcription has been shown to be up-regulated by IFNα and by inducers of IFN-α (23). Whereas IL-15 mRNA is expressed abundantly by many tissues (27), protein production has been identified only in culture supernatants of bone marrow stromal cells and activated monocytes (22, 28). It is likely that the IL-15 produced in the lung after infection was made by resident or infiltrating macrophages in response to infection and/or to type I IFN. This normal IL-15 induction was absent in both Stat1−/− and IFNAR−/− macrophages in vitro and in infected lungs. IL-15 has been shown to play an essential role in NK cell development and activation as well as in T cell proliferation and homing (29, 30).

Although cytokotoxicity by NK cells does not appear to be essential in protection from influenza virus (31), a role as cytokine producers may be important for resolution of disease. In addition, other IL-15 effects may be important for T cell differentiation, migration, and function in the setting of acute viral infection. Recent studies showing enhanced IFN-γ synthesis by IL-15-treated CD4+ lymphocytes lend support to its role as a T cell modulator (32).

Another cytokine lacking in infected lungs from Stat1−/− mice was IL-10 which appeared late during infection of wild-type animals. Although IL-10 production is usually associated with a Th2 bias, studies of cytokine production profiles in mediastinal lymph nodes of wild-type mice infected with influenza virus showed a concurrent synthesis of IL-2, IFN-γ, and IL-10 (24). We do not know which population of responding cells within the lung produced IL-10: IL-10 can be produced by CD4 cells, CD8 cells, B cells, and macrophages (24, 25). Nor do we know why there is a lag in its appearance in the lung. We observed induction of IL-10 mRNA synthesis in infected wild-type and IFN-γ−/− macrophage cultures, but not in cells derived from IFNAR−/− or Stat1−/− mice (Fig. 4). The similar lung IL-10 mRNA profiles in all but the Stat1−/− animals suggests either a nonmacrophage source at the site of infection, at least in the case of IFNAR−/− animals, or that tissue macrophages in the lung respond differently than the bone marrow-derived cells tested in vitro. It is possible that the presence of IL-10, which can act as a down-regulatory cytokine, serves to blunt somewhat the proinflammatory response to influenza virus in IFNAR−/− animals, resulting in milder inflammation than observed in Stat1−/− mice. Although the lung pathology in IFNAR−/− animals was exacerbated compared with wild-type or IFN-γ−/− mice, it was consistently less severe than that seen in Stat1−/− or IFNAR/Gr−/− knockouts. The preservation of type II
IFN signaling may also act to modulate inflammation in the lungs of IFNAR-/- mice relative to Stat1-/- animals. Therefore, although the Th2-predominant T cell response observed in Stat1-/- mice after influenza virus infection may partially account for the altered course of their disease, the absence of IFN-γ signaling alone is not sufficient to perturb the primary immune response to virus infection. Additionally, although Th2 predominance was observed in the progression of disease in Stat1-/- animals, a substantial population of IFN-γ-secreting CD8 cells was also present in the Stat1-/- mice, as were virus-specific cytotoxic T cells competent for virus elimination. This differs from the Th2 bias that can be produced by exogenous IL-4 administration during influenza virus infection which resulted in a delayed mark in viral clearance (33). Thus, the physiological defects resulting from the absence of Stat1 appear to be at least 2-fold. The first is an inappropriate production of Th2-type cells, cytokines, and Ig, apparent due to an inability to prevent Th2 cell differentiation and/or proliferation normally mediated by both type I and type II IFN. The second defect is the marked recruitment of proinflammatory macrophages, neutrophils, and eosinophils to the site of infection, a defect due substantially to the loss of type I IFN responsiveness and not due to the Th2 bias alone. This disease pattern is remarkably reminiscent of vaccine-enhanced respiratory syncytial virus disease and vaccine-induced atypical measles illness of children where an inappropriate Th2-like response to some vaccine preparations produces a debilitating inflammatory response rather than cell-mediated viral immunity (34, 35). Disregulated IFN production and/or responsiveness could be a factor in these syndromes.

IL-12- and Stat4-deficient mice also exhibit impaired Th1 and enhanced Th2 responses (36–39). Coupled with the results reported here, these polarized responses suggest that IL-12 is required for Th1 differentiation whereas IL-12-, type I IFN-, and type II IFN-mediated responses work in concert for suppression of Th2 cells. The finding that loss of the suppression of Th2 cells mediated by IFN-γ (40) does not result in exacerbated influenza virus-induced pathology implicates IFN-α as an important coordinator of both innate and specific responses to virus infection. Recent observations that either IL-12 or type I IFN, but not type II IFN, are capable of inducing the IL-12 receptor β2-chain and therefore IL-12 responsiveness on human T lymphocytes (41) lend support to this hypothesis as does the requirement of type I IFN for induction of IL-15 and IL-10, additional cytokines potentially important for disease resolution. There may also be an essential role for Stat1-mediated responses in immunoregulation by cytokines other than the IFNs, for example, in some responses to IL-12 (42). Along with recent studies of mice deleted for other Stat genes (38, 39, 43, 44), the results reported here show the intimate relationship among Stat gene function, the functional diversity of lymphocytes, and the development of innate and acquired immunity to pathogens.

Acknowledgments

We thank P. Palese (Mount Sanai School of Medicine) for suggesting experiments with influenza virus and for advice, support, and encouragement; G. Inghirami and J. Hirst (New York University) for help with flow cytometry; R. Schreiber (Washington University) and M. Aguet (Lausanne) for gifts of IFN-resistant mice; W. Paul (National Institutes of Health), S. Haba, and A. Nisonoff (Brandeis University) for gifts of reagents; and J. Thorbecke (New York University), P. R. Johnson (Ohio State University), and R. Gimeno (New York University) for helpful discussions and critical comments on the manuscript.

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