The Journal of Immunology
RESEARCH ARTICLE | NOVEMBER 01 2010

IL-22 Induces an Acute-Phase Response

Spencer C. Liang; ... et. al

J Immunol (2010) 185 (9): 5531–5538.
https://doi.org/10.4049/jimmunol.0904091

IL-22 Induces an Acute-Phase Response

Spencer C. Liang,*[,1,2] Cheryl Nickerson-Nutter,*[,1] Debra D. Pittman,[†] Yijun Carrier,* Debra G. Goodwin,* Kathleen M. Shields,[†] Andre-Jean Lambert,[‡,3] Scott H. Schelling,[‡] Quintus G. Medley,* Hak-Ling Ma,* Mary Collins,* Kyriaki Dunussi-Joannopoulos,* and Lynette A. Fouser*

IL-22 is made by a unique set of innate and adaptive immune cells, including the recently identified noncytolytic NK, lymphoid tissue-inducer, Th17, and Th22 cells. The direct effects of IL-22 are restricted to nonhematopoietic cells, its receptor expressed on the surface of only epithelial cells and some fibroblasts in various organs, including parenchymal tissue of the gut, lung, skin, and liver. Despite this cellular restriction on IL-22 activity, we demonstrate that IL-22 induces effects on systemic biochemical, cellular, and physiological parameters. By utilizing adenoviral-mediated delivery of IL-22 and systemic administration of IL-22 protein, we observed that IL-22 modulates factors involved in coagulation, including fibrinogen levels and platelet numbers, and cellular constituents of blood, such as neutrophil and RBC counts. Furthermore, we observed that IL-22 induces thymic atrophy, body weight loss, and renal proximal tubule metabolic activity. These cellular and physiological parameters are indicative of a systemic inflammatory state. We observed that IL-22 induces biochemical changes in the liver including induction of fibrinogen, CXCL1, and serum amyloid A that likely contribute to the reported cellular and physiological effects of IL-22. Based on these findings, we propose that downstream of its expression and impact in local tissue inflammation, circulating IL-22 can further induce changes in systemic physiology that is indicative of an acute-phase response. ***The Journal of Immunology*****, 2010, 185: 5531–5538.**

Interleukin-22 is a type II cytokine that was identified ~10 years ago as a result of its low homology to IL-10 (1). Although originally reported to be made by activated human Th1 and conventional NK cells (2), studies over the past few years have demonstrated that IL-22 is highly dependent on IL-23 for its production from Th17 and γ δ T cells, as well as the novel noncytolytic mucosal NK and lymphoid tissue-inducer cells (3–6). Most recently, IL-22 has been found to be expressed from novel skin-homing Th22 cell lineages (7–10). IL-22 signals into a cell via a complex that it forms with IL-22R and IL-10R2 (11–14). These receptor subunits belong to the cytokine receptor family 2, the members being receptors for type II cytokines and including the IFNs (15). Although IL-10R2 is expressed ubiquitously, almost all data suggest that IL-22R is expressed only on epithelial cells and some fibroblasts (16, 17). This restricted expression of IL-22R indicates that IL-22 acts directly on nonimmune cells in peripheral tissues to exert its functions.

IL-22 has a critical role in modulating local inflammation in certain organs. IL-22 contributes to the maintenance of mucosal barrier integrity and generation of a protective inflammatory response against certain extracellular bacterial pathogens (18, 19). IL-22 alone is able to induce skin hyperplasia and epidermal wound healing (10, 20). IL-22 is also involved in noninfectious inflammatory disease states and can play either a pathogenic (i.e., skin, joints) or protective (i.e., gut, liver) role (21–26). The gross affect of IL-22 signaling is dependent on the cellular and biochemical milieu in which IL-22 effects gene expression. IL-22 has the capacity, particularly in synergy with other cytokines, to induce gene expression for antimicrobial peptides, chemokines, matrix metalloproteinases, cytokines, and acute-phase proteins from epithelial cells in the skin, gut, lung, or liver (10, 18–20, 27).

The liver is required for the constitutive production of blood proteins and is the major source of acute-phase proteins (e.g., serum amyloid A [SAA], fibrinogen, C-reactive protein). With uncontrolled local tissue inflammation, IL-6 and other family members, TNF-α, and IL-1β circulate to the liver and induce the production of these acute-phase proteins that subsequently function within, and are indicative of, an acute-phase response (28–30). In the context of a typical infection or trauma, the acute-phase response is a transient and systemic modulation of physiological systems that together facilitate an immune response, resolve the local inflammation, repair the injured tissue, and re-establish homeostasis (28–30). In the context of dysregulated inflammation and autoimmunity, the acute-phase response is chronic, with the potential for the inflammation to become more systemic. Early studies suggested that IL-22 may induce an acute-phase response. Dumoutier et al. (31) identified an IL-22–dependent induction of acute-phase protein gene expression from the HepG2 hepatocellular carcinoma-derived cell line and from the liver. Wolk et al. (17) subsequently reported increased levels of circulating SAA postadministration of IL-22.

In this study, we report that sustained IL-22 exposure leads to an acute-phase response that is detected at the blood (i.e., modulation of acute-phase proteins and blood cells), organ (i.e., thymic

*Inflammation and Immunology Research Unit and [†]Biocorrection Research Unit, Pfizer Biotherapeutics Research and Development, Cambridge, MA, 02140; and [‡]Pfizer Global Drug Safety Research and Development, Andover, MA, 01810

[1]S.C.L and C.N.-N. contributed equally to the science described in this work.

[2]Current address: Takeda San Francisco, South San Francisco, CA.

[3]Current address: Charles River Laboratories, Montreal, Quebec, Canada.

Received for publication December 21, 2009. Accepted for publication August 23, 2010.

Address correspondence and reprint requests to Dr. Lynette A. Fouser, Principal Research Scientist II, Inflammation and Immunology Research Unit, Pfizer Biotherapeutics Research and Development, 200 CambridgePark Drive, Cambridge, MA 02140. E-mail address: lynette.fouser@pfizer.com

Abbreviations used in this paper: AdIL-22, IL-22-expressing adenoviral vector; AdGFP, GFP-expressing adenoviral vector; b2m, β_2-microglobulin; G, glomerulus; SAA, serum amyloid A; WT, wild-type.

involution), and whole animal (decrease in body weight) level. We further show that a single i.p. injection of IL-22 induces the hepatic production of circulating SAA, independent of IL-6 or TNFR1 signaling, and the transient mobilization of neutrophils that is associated with the rapid induction of hepatic gene expression for CXCL1, a neutrophil chemoattractant. The data of this study support the proposal that circulating IL-22, subsequent to its local expression in the context of tissue inflammation, is able to contribute also to the induction of systemic physiological processes, at least in part via its action in the liver.

Materials and Methods

Reagents

IL-22 (AdIL-22)- and GFP (AdGFP)-expressing adenoviral vectors were generated by inserting a mouse IL-22 or GFP cDNA fragment downstream of the CMV immediate early promoter and enhancer into a plasmid that contains 0–1 and 9–16 map units of Ad5 virus. These vectors were linearized and individually used with a linearized helper vector, which contains 9–36 map units, to cotransfect the human embryonic kidney cell line HEK293 that expresses adenoviral E1a constitutively (32, 33). The resultant replication-defective adenovirus, derived by intracellular homologous recombination and packaging, contains a deletion of E1a and E3. This virus was then amplified by further infection of 293 cells. Adenovirus used for administration to mice was released from infected 293 cells by three cycles of freeze thawing, purified by two cesium chloride centrifugation gradients, and dialyzed against PBS (pH 7.2) at 4°C. Following dialysis, glycerol was added to a concentration of 10%, and the virus was stored at −80°C until use. Mouse IL-22 was generated using methods previously described and had <1 endotoxin unit/mg (14, 34).

Mice

C57BL/6J, IL-6 deficient (B6;129S2-Il6tm1Kopf/J), and TNFR1-deficient (B6;129S-Tnfrsf1atm1Imx) female mice were obtained from The Jackson Laboratory (Bar Harbor, ME). All mice were used between 8–12 wk of age and housed in strict accordance with Pfizer Research Institutional Animal Care and Use Committee regulations.

Adenoviral infections

A single dose of 5×10^{10} particles of recombinant adenovirus that expressed mouse IL-22 or GFP, the latter as the control, was injected into the tail vein of female C57BL/6J mice, age 7 to 8 wk, four to eight mice per group depending on the experiment. Mice from an experimental group were sacrificed at a given time point postinjection. Blood was collected by cardiac puncture for hematological and serum chemistry and by retro-orbital sinus puncture for differential counts on blood smears, all performed at Ani Lytics (Gaithersburg, MD). Tissue was harvested, fixed in formalin, and stained with H&E for histopathology evaluation at Pathology Associates International (Wilmington, MA) or internally.

IL-22 i.p. administration

C57BL/6J mice were injected with 25 μg rIL-22 or vehicle control (low-endotoxin PBS) via the i.p. route of administration. Blood and liver were harvested at 0.5, 1, 3, 6, and 24 h postadministration and analyzed. Neutrophil counts in the blood were determined using Cell-Dyn hematology analyzer (Abbott Diagnostics, Vienna, Austria). SAA and CXCL1 were quantified by SAA (Invitrogen, Carlsbad, CA) and CXCL1 (R&D Systems, Minneapolis, MN) ELISA, respectively, following the manufacturer's directions. RNA from the liver was prepared using the Ribopure RNA isolation kit (Ambion, Austin, TX). Quantitative RT-PCR was performed by TaqMan (Applied Biosystems, Foster City, CA) using prequalified primer/probes (Applied Biosystems). Concentrations of IL-6 and TNF-α were determined by the Inflammation CBA kit (BD Pharmingen, San Diego, CA).

Results

IL-22 delivered by adenoviral infection causes hematological changes, loss of body weight, thymic atrophy, and structural changes to the renal proximal tubules

To study potential systemic roles downstream of its expression, IL-22 was first ectopically expressed in C57BL/6J mice using a replication-defective adenovirus. Four days after AdIL-22 administration, 35–95 ng/ml IL-22 was detected in serum from a given mouse; with AdGFP treatment, ≤0.005 ng/ml IL-22 was detected in the serum (data not shown). Ki et al. (35) have recently reported the first use of adenovirus for the ectopic expression of IL-22, administering 2×10^8 PFU and detecting 4.5 ng/ml circulating IL-22 after 2.5 d. The 10–20-fold higher levels that we observed may be due to the use of a higher adenoviral dose (5×10^{10} particles) and a later readout (4 d) of serum IL-22 levels.

We examined the expression of acute-phase proteins up to 2 wk after adenoviral infection. Whereas AdGFP induced the production of SAA that was detected in the blood as early as 1 d postinfection, this expression of SAA resolved by day 3 (Fig. 1*A*). In contrast, AdIL-22 sustained high levels of circulating SAA on days 3, 7, and 14 postinfection. SAA in the blood, after AdIL-22 infection, peaked at a mean concentration of 770 μg/ml on day 7, an 85-fold induction relative to the level observed after AdGFP infection. Fibrinogen, another acute-phase protein, was also enhanced in the blood of mice administered AdIL-22, starting as early as day 1 and sustained on days 3 and 7 (Fig. 1*B*). AdIL-22 infection induced a 4.6-fold induction of fibrinogen on day 7 that was statistically significant relative to AdGFP infection. Again, AdGFP initially induced fibrinogen expression that began to resolve by day 3. Whereas the expression of most acute-phase proteins is elevated, albumin decreases during an acute-phase response (36). AdIL-22–infected mice have a subtle, yet statistically significant and consistent, decrease in serum albumin on day 7 compared with AdGFP-infected control mice that was no longer significant on day 14 (Fig. 1*C*). These observations demonstrated that exposure to IL-22 for 2 wk, using a replication-defective adenovirus for ectopic expression, resulted in the modulation of certain blood proteins indicative of an acute-phase response.

We also examined the effects of AdIL-22 infection on levels of circulating blood cells. Mice treated with AdIL-22 had a statistically significant 1.4- and 2-fold increase in blood platelets, compared with AdGFP-treated groups, on days 7 and 14, respectively,

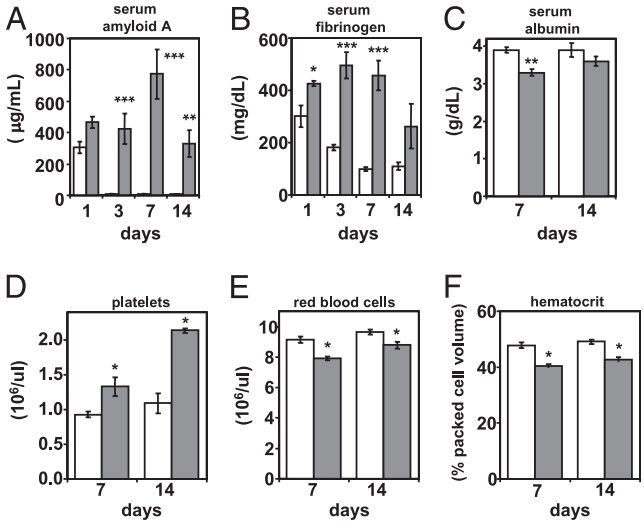

FIGURE 1. Infection with replication-defective AdIL-22 modulates acute-phase proteins and cellular constituents in the blood. Group means are shown for SAA (*A*), fibrinogen (*B*), and albumin in the serum (*C*) and platelets (*D*), RBCs (*E*), and hematocrit (*F*) in blood collected from mice on day 1, 3, 7, or 14 after mice were inoculated i.v., on day 0 with AdIL-22 (dark bars) or AdGFP (open bars). Data are representative of two to five experiments, depending on the parameter shown. Groups contained four to eight mice, depending on the study, with error bars indicating the SEMs and statistics derived from an unpaired *t* test. $*p \leq 0.05$; $**p \leq 0.01$; $***p \leq 0.005$.

after adenovirus administration (Fig. 1*D*). In addition, we detected a statistically significant 13 and 14% decrease in circulating RBCs on days 7 and 14, respectively, in the AdIL-22–treated mice (Fig. 1*E*). Consistent with this, statistically significant decreases were also detected in both the serum hematocrit (Fig. 1*F*) and hemoglobin (data not shown). We observed increased numbers of segmented neutrophils in the blood, although not always statistically significant (data not shown). Taken together, these hematological changes, observed in mice infected with AdIL-22, suggested that IL-22 is able to induce an acute-phase response.

A trend toward decreased whole body weight for AdIL-22–treated mice, relative to AdGFP-treated, was observed 7 d after viral administration. This differential in body weight was statistically significant by 14 d, with a group mean loss of 9% determined for AdIL-22–treated mice, relative to AdGFP-treated (Fig. 2*A*). A loss in body weight is also indicative of an acute-phase response (37).

GFP detection in tissue from mice infected with AdGFP demonstrated that the liver is the major organ of adenoviral infection (data not shown), consistent with previous reports regarding adenoviral tissue infectivity (38). Whereas IL-22 has the ability to be hepatoprotective and hepatoproliferative (23, 26, 39, 40), AdIL-22 administration did not induce an increase in liver weight relative to the AdGFP treatment (Fig. 2*B*; noting that each symbol is a mean from a study, with the horizontal bar indicating the mean of the means from four or five studies). The reproducible decrease in organ weight between day 7 and day 14 is probably due to resolution of the replication-defective adenoviral-induced inflammation including edema of the liver (J. M. Wilson, personal communication). Microscopic examination of the liver indicated a slight to mild single-cell hepatocellular necrosis with mononuclear cell, mixed cell, or neutrophilic infiltrates observed in livers from AdIL-22– and AdGFP-treated mice sacrificed on day 7 and 14 postinfection (data not shown). The severity and incidence of liver inflammation was comparable between the two groups over several studies. We conclude that the ectopic hepatic expression of IL-22 did not affect the adenoviral-induced liver inflammation.

Whereas IL-22 was primarily expressed in the liver, we also looked for effects of AdIL-22 infection in other organs. We and others have reported previously that IL-22 induces keratinocyte

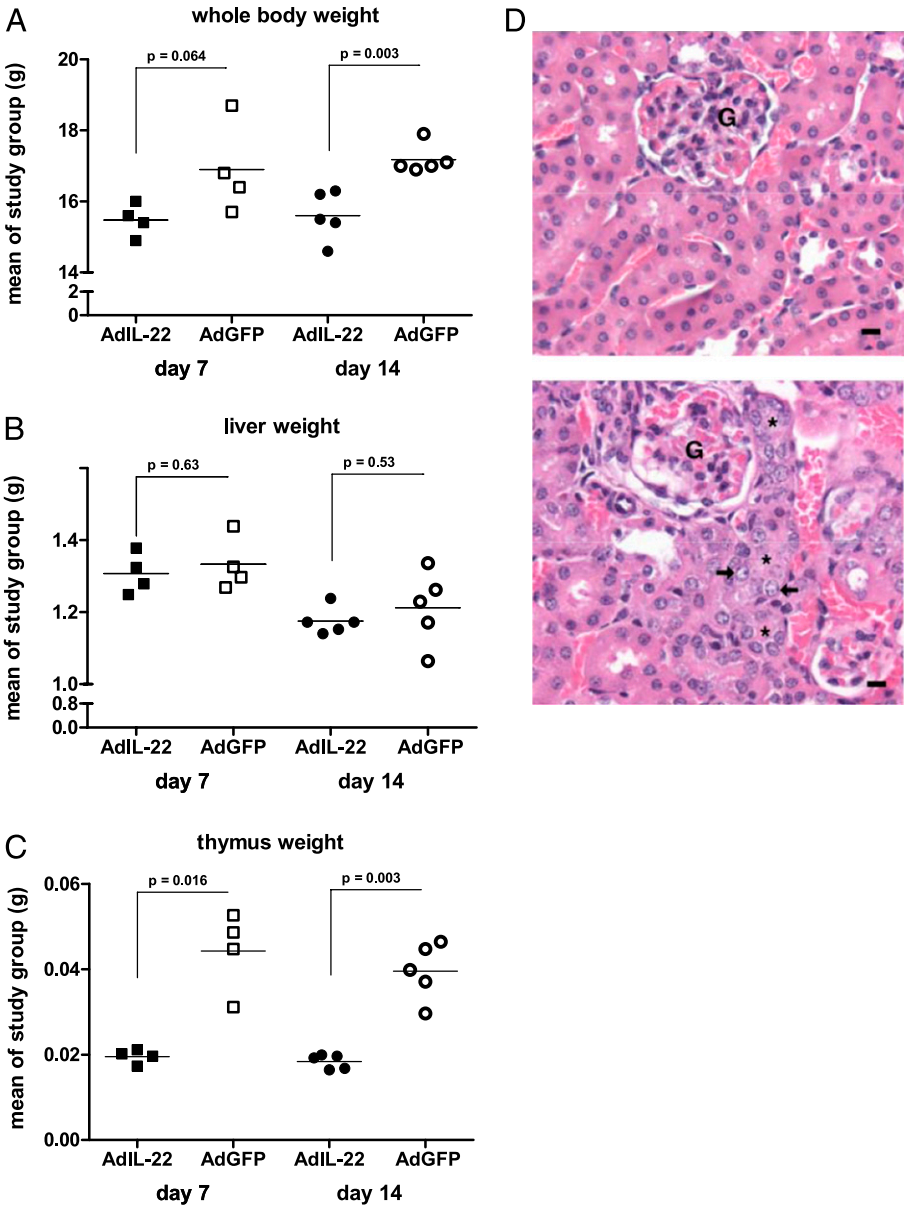

FIGURE 2. Infection with replication-defective AdIL-22 induces loss of body weight, thymic atrophy, and changes to renal proximal tubules. Group mean body (*A*), liver (*B*), and thymus (*C*) weights are shown for AdIL-22 and AdGFP-infected mice at days 7 (four studies) and 14 (five studies) after receiving a single i.v. injection of the replication-defective adenovirus on day 0. Groups contained four to eight mice, depending on the study, with *p* values derived from a paired *t* test. *D*, Representative photomicrographs from five studies of the renal cortex from female C57BL/6J mice 14 d after receiving a single i.v. injection of AdGFP (*top panel*) or AdIL-22 (*bottom panel*). The AdGFP-exposed kidney is within normal limits. Several proximal convoluted tubules (∗) within the kidney from AdIL-22–treated mice are lined by epithelial cells that have slightly enlarged, vesicular nuclei (arrows) and basophilic cytoplasm compared with other tubular epithelial cells. H&E stain, original magnification ×20. Scale bar, 10 μm. G, glomerulus.

hyperplasia and that blockade of IL-22 prevents the progression of psoriasis-like skin inflammation (19–21). With IL-22 administered by adenovirus, we did not observe any macroscopic changes in the skin at necropsy on day 7 or 14; we did not evaluate the skin microscopically. We did not detect any distinct microscopic findings in the brain, spinal cord, lung, heart, spleen, submaxillary lymph nodes, adrenal glands, pancreas, ovaries, vertebrae, or bone marrow from mice administered AdIL-22 compared with AdGFP (data not shown). AdIL-22–related effects were, however, observed in the thymus and kidney.

Lymphoid depletion in the thymus was detected microscopically in all groups of mice and occurred with a higher incidence on days 7 and 14 in mice receiving AdIL-22 (data not shown). Described as generalized thymic atrophy, there was an observed decrease in the number of cortical and medullary lymphocytes in thymuses from AdIL-22 treated mice relative to GFP treated. These microscopic findings in the thymus correlated with lower absolute (Fig. 2*C*) and relative (data not shown) thymus weights from mice treated with AdIL-22 compared with AdGFP. The group means for absolute thymus weights from AdIL-22–treated mice on days 7 and 14 after viral administration were 56 and 54% lower, respectively, than the means from AdGFP-treated mice. These data indicated that sustained IL-22 exposure delivered by adenovirus induced thymic atrophy, which is also a characteristic of an acute-phase response (41).

Slight to mild multifocal tubular basophilia was observed in the renal cortex on days 3, 7, and 14 in AdIL-22–treated mice (Fig. 2*D*). Basophilia in a tissue refers to blue-purple staining of nucleic acid-containing constituents by the basic dye hematoxylin (42). This heightened staining of nucleic acid is indicative of cellular proliferation and metabolic changes associated with tissue regeneration subsequent to injury (43, 44). Affected proximal convoluted tubules were lined by epithelial cells that had slightly enlarged vesicular nuclei and basophilic (i.e., hematoxyphilic) cytoplasm compared with tubular epithelial cells in other segments of the nephron (Fig. 2*D*).

AdIL-22 studies demonstrated that sustained IL-22 exposure is able to induce blood, organ, and whole body effects indicative of an acute-phase response. We addressed whether the adenoviral vehicle itself contributed to the overall effects of AdIL-22 by evaluating in one study the impact of daily injections of IL-22 protein. Overall, the modulation of acute-phase proteins, blood cells, and organ and body weight changes observed with AdIL-22 were recapitulated with daily cytokine injections over a 2-wk period (data not shown). In particular, 25 μg IL-22 daily, administered either s.c. or i.v., induced a group mean 8% decrease in body weight and a 60% decrease in absolute thymus weight, both relative to daily vehicle injections. Renal tubular basophilia was also observed in the kidneys of mice that were administered recombinant mouse IL-22 daily for 2 wk (data not shown). Whereas the adenoviral delivery may have contributed to the intensity of certain sustained effects (i.e., circulating SAA), we conclude that IL-22 alone is able to sustain an acute-phase response over several days.

IL-22 induces the production of SAA independent of IL-6 or TNFR1 signaling

To study further acute systemic effects of IL-22, we administered a single 25-μg dose of IL-22 to mice by i.p. injection and examined the blood and liver. As early as 3 h postadministration of cytokine, IL-22 induced a statistically significant 2.4-fold increase, relative to PBS, of circulating SAA protein that increased to 17-fold and 250-fold at 6 and 24 h, respectively (Fig. 3*A*). Because hepatocytes are the major source for acute-phase proteins (30), we evaluated SAA and other acute-phase protein gene expression in the liver after i.p. IL-22 or PBS administration. As early as 0.5 h posttreatment, the induction of SAA1 transcript was detected in some of the IL-22–treated animals with statistically significant 42-fold and 3.3-fold increases observed at 1 and 3 h, respectively (Fig. 3*B*). This induction of SAA liver transcript and circulating protein by IL-22 is consistent with previously reported results (17, 31). We also observed that IL-22 significantly enhanced fibrinogen transcript in the liver as early as 1 h postinjection (Fig. 3*B*). Although not statistically significant, there was also a trend toward increased gene expression for haptoglobin (Fig. 3*B*). IL-22 did not modulate albumin transcript levels in the liver (Fig. 3*B*). Together, these data indicated that IL-22 induces the expression of acute-phase proteins from the liver as soon as 1 h after its i.p. administration.

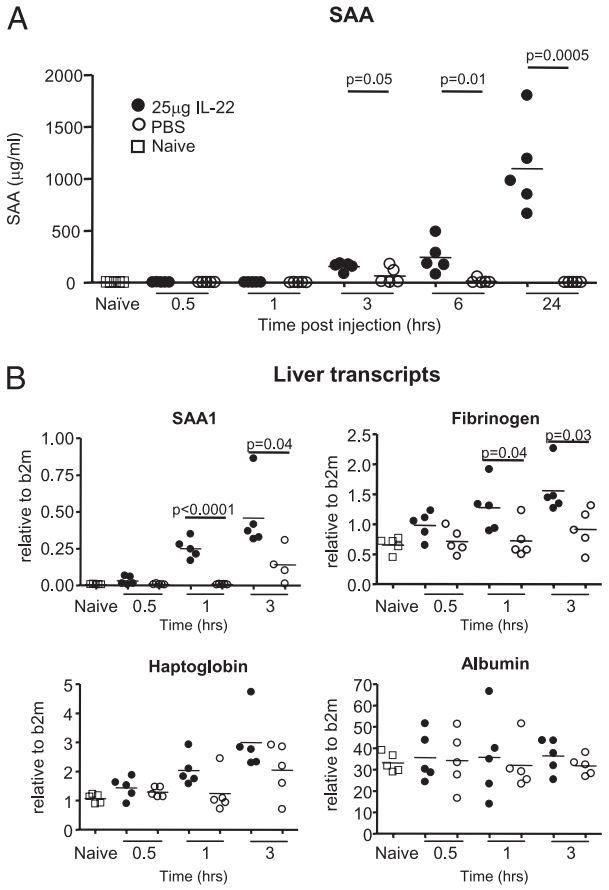

FIGURE 3. A single injection of IL-22 induces SAA and fibrinogen A from the liver. *A*, Mice were administered 25 μg IL-22 protein or PBS via i.p. injection. Groups of mice, $n = 5$, were sacrificed at the indicated time points, blood harvested, and SAA concentrations in serum determined by ELISA. *B*, Livers from mice administered IL-22 or PBS, as indicated above, were snap frozen and processed for RNA. Quantitative PCR for SAA1, fibrinogen A, haptoglobin, and albumin cDNA after reverse transcription were performed and relative amounts of each product calculated with normalization to β_2-microglobulin (b2m). Data are representative of two to four experiments, depending on the parameters shown, with *p* values derived from an unpaired *t* test.

Although injection of IL-22 is sufficient to induce SAA, IL-22 may be doing this indirectly via the expression of another cytokine that then effects the expression of SAA from the liver. We determined that a single injection of IL-22 does not modulate IL-6, TNF-α, and IL-1β gene expression in the liver or kidney (data not shown) or IL-6 and TNF-α protein in the blood (Fig. 4*A*). We also determined that IL-22 is able to induce the production of circulating SAA in IL-6– or TNFR1-deficient mice to the same levels

observed in wild-type controls (Fig. 4*B*). These data suggest that IL-22 is able to act via its receptor on hepatocytes to directly modulate SAA transcription and translation from hepatocytes in the liver.

IL-22 induces transient neutrophil mobilization and CXCL1 in the blood

We observed that a single i.p. injection of IL-22 induced a transient, statistically significant 2-fold increase in neutrophil counts in the blood 1 h after cytokine administration (Fig. 5*A*). We detected a statistically significant 14-fold transient increase in CXCL1 in the serum at 1 h postadministration (Fig. 5*B*) and determined that CXCL1 transcripts in the liver were significantly enhanced (i.e., 55-fold) by 0.5 h postinjection (Fig. 5*C*). In consideration of the transient increase of neutrophils and the injection site, we examined the peritoneal lavage of mice at 24 h but did not detect an increase in neutrophil levels in the IL-22–treated mice relative to vehicle. In the context of adenovirus delivery, in which there was a trend (i.e., not always statistically significant within a given study; data not shown) toward an increase in blood neutrophils, microscopic examination did not indicate an increased extravasation of neutrophils to livers of AdIL-22–treated mice relative to AdGFP. Although we do not know the target organ for the neutrophils, we demonstrated with our data that IL-22 can induce neutrophil mobilization that is tightly associated with the expression of the neutrophil chemoattractant CXCL1 from the liver and its circulation in the blood.

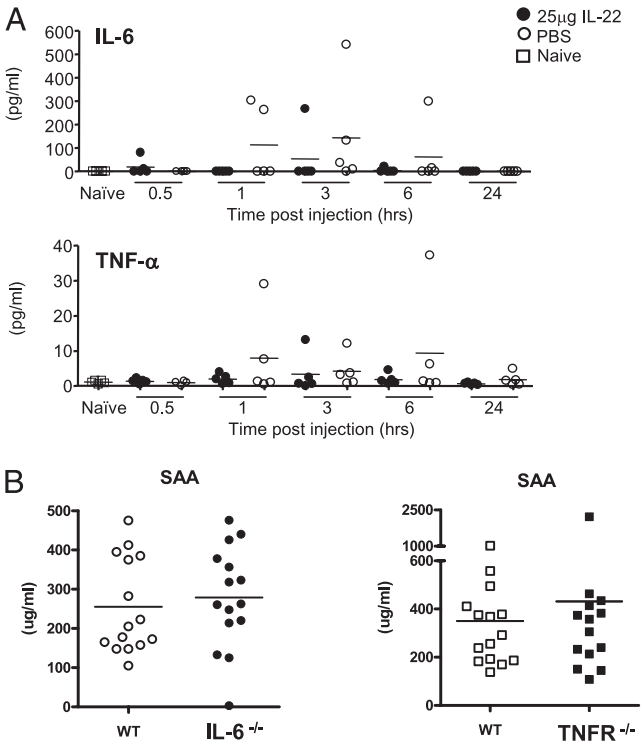

FIGURE 4. IL-22 induction of SAA is independent of IL-6 and TNFR1. *A*, Mice were i.p. injected with 25 μg IL-22 protein or PBS. Groups of mice, $n = 5$, were sacrificed at the indicated time points, blood harvested, and IL-6 and TNF-α concentrations in serum determined by bead capture assay. Data are representative of three experiments. *B*, Wild-type (WT), IL-6–deficient (IL-$6^{-/-}$), and TNFR1-deficient (TNFR$1^{-/-}$) mice were injected with 25 μg IL-22. Mice were bled at 6 h postinjection and levels of SAA in serum determined by ELISA. $n = 15$ mice/group. Data are representative of two experiments.

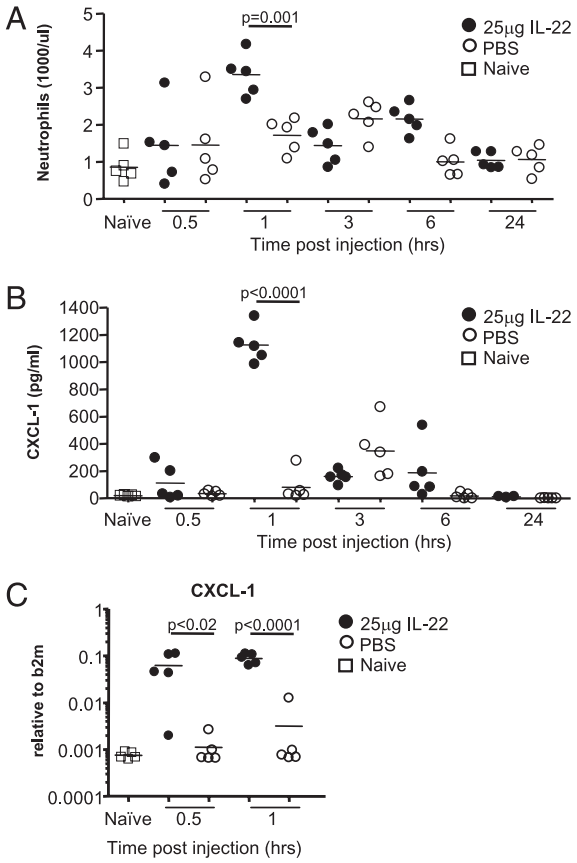

FIGURE 5. A bolus injection of IL-22 induces transient circulating CXCL1 and neutrophil mobilization. *A*, Mice were i.p. injected with 25 μg IL-22 or PBS with groups subsequently sacrificed at several time points, blood collected, and neutrophil numbers quantified using a CELL-DYN^{R}. The same mice as above were also evaluated for CXCL1 in the serum by ELISA (*B*) and transcript in the liver, relative to b2m, by quantitative PCR (*C*). The data in *A* and *B* are representative of four experiments, with *C* representative of data from two of these experiments. The *p* values were derived from an unpaired *t* test.

Discussion

IL-22 is made by Th17, noncytolytic NK, lymphoid tissue-inducer, and skin homing Th22 cells (3–10). Although produced by these novel innate and adaptive immune lineages, IL-22 signals via its receptor only on the surface of epithelial cells and some fibroblasts (11–14). IL-22 is therefore proposed to function locally to modulate tissue inflammation (1). We demonstrate in this paper that IL-22 also has the ability to induce systemic effects in the blood, certain organs, and whole body. We report for the first time, to the best of our knowledge, that IL-22 induces hepatic gene expression for fibrinogen and CXCL1, modulates circulating levels of fibrinogen, CXCL1, neutrophils, platelets, and RBCs, produces SAA independent of either IL-6 or TNFR1 signaling, and causes thymic atrophy and body weight loss. Together, the effects that we observe are indicative of an acute-phase response (28–30). In addition, we report that IL-22 induces renal tubular basophilia. Collectively, the data of this study support the proposal that IL-22 can modulate systemic processes as a result, in part, of its circulation to and induction of gene expression in the liver.

The liver was the first organ shown to be grossly affected by IL-22 in the context of local tissue inflammation. Radaeva et al. (23) and Pan et al. (45) determined that IL-22 induces antiapoptotic and mitogenic gene expression from hepatocytes in vitro and in the liver. They proposed a functional correlation of this gene ex-

pression with the ability of IL-22 to protect the liver from carbon tetrachloride, Fas ligand, and ConA-induced injury. Protective effects of IL-22 in the context of hepatitis were subsequently confirmed by Zenewicz et al. (26) using IL-22–deficient mice. Brand et al. (39) proposed that IL-22 is able to maintain metabolic activity of primary human hepatocytes in vitro with IL-22 transcription induced in the liver in the context of human hepatitis (46). Ren et al. (40) have most recently shown that IL-22 plays a role in liver regeneration subsequent to partial hepatectomy. Thus, the local induction of gene expression by IL-22 is proposed to protect the liver from inflammation. Interestingly, we observed microscopically that the ectopic expression of IL-22 in the liver does not appear to affect the mild inflammation that is induced by a replication defective adenovirus. In addition, AdIL-22 did not induce heightened proliferation of hepatocytes that was detectable microscopically or by a change in liver weight relative to AdGFP-treated mice. We conclude that the local effects of IL-22 in the context of a replication defective adenoviral infection of the liver are distinct from the hepatoprotective effects of IL-22 observed subsequent to viral, chemical, or surgical injury to the liver.

We do propose that IL-22, either produced in or circulating to the liver, can act directly on the liver to effect hepatic gene expression that has systemic ramifications. In this study, we demonstrate that CXCL1 transcript is induced in the liver in vivo by 30 min (Fig. 5*C*) and fibrinogen A and SAA1 by 1 h (Fig. 3*B*) after a single i.p. injection of IL-22. Furthermore, the proposed resultant increase in circulating SAA is independent of either IL-6 or TNFR1 signaling (Fig. 4*B*). We also did not detect an increase in TNF-α, IL-6, or IL-1β transcript in the liver or kidney (data not shown) or increased levels of TNF-α or IL-6 protein in the blood (Fig. 4*A*). Consistent with our findings, Dumoutier et al. (31) demonstrated that IL-22 induces SAA gene expression in the HepG2 hepatocellular carcinoma-derived cell line in vitro. Cycloheximide, a protein synthesis inhibitor, did not inhibit this SAA transcription, suggesting that IL-22 directly induces gene expression of SAA from this cell line. The observed rapid in vivo onset of IL-22–mediated hepatic gene expression, accompanied by a lack of TNF-α, IL-6, and IL-1β transcription and/or protein, supports the conclusion that IL-22 is acting directly to induce the expression of blood proteins.

Acute-phase proteins, induced in the context of inflammation, have been demonstrated or hypothesized to facilitate systemic and physiologic processes. We report on a temporal relationship between IL-22, CXCL1, and neutrophils. A single i.p. injection of IL-22 induces CXCL1 transcription in the liver by 30 min, with a transient increase in circulating CXCL1 protein and neutrophils detected by 1 h (Fig. 5). G-CSF, an inducer of neutrophil development, and CXCL2 and CXCL5, mobilizers of neutrophils, were undetectable in the serum (data not shown). We therefore propose that CXCL1 is the acute-phase protein and chemokine that leads to the transient elevation of circulating neutrophils after a single administration of IL-22. Our observations suggest that exposure of the liver to circulating IL-22, subsequent to its production elsewhere by tissue-resident innate and adaptive immune cells, may be a mechanism for enhanced systemic immunity.

IL-22 also induces hepatic gene expression of SAA, with 1 mg/ml levels of circulating SAA detected in the blood after a single injection of IL-22 (Fig. 3) and elevated levels maintained with sustained exposure to IL-22 (Fig. 1*A*, data not shown). SAA is a lipophilic protein and the major acute-phase protein in mice (47). Although formyl peptide receptor-like 1 was originally defined as the cell surface receptor for SAA (48), Cheng et al. (49) have recently determined that SAA also signals via TLR2. As SAA has pleiotropic functions, including the direct opsonization of certain bacteria and viruses, binding and transport of cholesterol, cell trafficking and chemotaxis, and induction of cytokine and chemokine gene expression (47), it is possible that IL-22–induced SAA is mediating effects of systemic IL-22 exposure.

A relationship between SAA and IL-22 in a disease prevention setting has recently been reported. Misse et al. (50) described a group of individuals whom have been repeatedly exposed to HIV-1 and yet are still uninfected with virus. These individuals have elevated levels of an SAA cleavage product in their blood. PBMC-derived, activated $CD4^+$ T cells from these uninfected yet virally exposed individuals contain 13- and 26-fold higher levels of IL-22 transcript compared with corresponding cells from healthy and HIV-infected individuals, respectively. No changes in transcript levels for IL-6, TNF-α, and IL-1β among these patient groups were observed. As rSAA was shown to downmodulate the CCR5 HIV-1 coreceptor on the surface of target cells and thereby block viral infectivity in vitro, the authors propose that the increased production of IL-22 by T cells in these individuals may contribute to the production of the novel SAA fragment and subsequent protection from viral infection.

With regard to intestinal homeostasis, Ivanov et al. (51) have recently demonstrated that commensal segmented filamentous bacteria induce both SAA expression and Th17 cell expansion in the terminal ileum. Concomitant in vitro mechanistic studies determined that SAA-induced IL-6 and IL-23 from intestinal dendritic cells enables the differentiation of Th17 cells, including the expression of IL-22. Thus, SAA and IL-22 have the potential to act in a positive feedback loop, amplifying the expression and function of each other.

SAA is also able to induce renal tubule formation in vitro with the in vivo expression of SAA associated with tubule generation in embryonic or ischemic kidneys (52). We demonstrate that sustained IL-22 exposure, delivered by adenovirus (Fig. 2*C*), induces some metabolic or proliferative activity in proximal tubules in the kidney. IL-22 may be acting directly on the renal proximal tubules, as IL-22R is expressed on epithelial cells and detected in the kidney. Alternatively, the IL-22–mediated renal alterations that we detected may be due to the induction of SAA by IL-22 and the subsequent direct effects of SAA on epithelial cells in the kidney.

Whereas IL-22 is proposed to act on epithelial cells in the context of its local production with tissue inflammation, including at mucosal barriers and in the skin, our observations suggest that IL-22 can also act distant from these sites of expression and subsequent to its circulation. We note that IL-22 is readily detected in the blood of animals, including humans, that present with inflamed tissue (21, 27). We propose that additional systemic functions of IL-22 will be defined as a result of its action in the liver and, potentially, in synergy with other cytokines, Taken together, IL-22, despite the lack of its receptor expression on immune cells, could have considerable indirect systemic effects on immunity and leukocyte function potentially as a result of its direct induction of SAA and other acute-phase proteins such as CXCL1 from the liver.

An acute-phase response is the liver-directed modulation of a variety of physiological systems, including coagulation and fibrinolytic, the goal being to facilitate an animal's return to whole-body homeostasis. With the initial definition of the highly expressed acute-phase proteins, the concept of an acute-phase response subsequently developed as a collection of biomarkers that superficially describe a very complex set of mechanisms driven by a variety of cytokines effects on the liver. Over the past 10 y, acute-phase proteins have received a fresh functional relevance and are used to measure systemic inflammation in an individual. Our science in this study considers the singular impact of IL-22 in

the liver with regard to an acute-phase response. We show that IL-22 can quickly induce biochemical mediators that can have substantial impacts on blood proteins, blood cells, immune cells, and peripheral tissues. Our data suggest that the impact of IL-22 on biological functions in vivo, through secondary mediators, extends beyond cells that express the IL-22 receptor.

Acknowledgments

We thank Khetemenee Lam for preparation of the mouse IL-22 and Marion Kasaian for a careful and thoughtful reading of the manuscript.

Disclosures

S.C.L. and A.-J.L. have no financial conflicts of interest. All other authors are currently employees of Pfizer.

References

1. Wolk, K., and R. Sabat. 2006. Interleukin-22: a novel T- and NK-cell derived cytokine that regulates the biology of tissue cells. *Cytokine Growth Factor Rev.* 17: 367–380.
2. Wolk, K., S. Kunz, K. Asadullah, and R. Sabat. 2002. Cutting edge: immune cells as sources and targets of the IL-10 family members? *J. Immunol.* 168: 5397–5402.
3. Liang, S. C., X. Y. Tan, D. P. Luxenberg, R. Karim, K. Dunussi-Joannopoulos, M. Collins, and L. A. Fouser. 2006. Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. *J. Exp. Med.* 203: 2271–2279.
4. Martin, B., K. Hirota, D. J. Cua, B. Stockinger, and M. Veldhoen. 2009. Interleukin-17-producing gammadelta T cells selectively expand in response to pathogen products and environmental signals. *Immunity* 31: 321–330.
5. Sutton, C. E., S. J. Lalor, C. M. Sweeney, C. F. Brereton, E. C. Lavelle, and K. H. G. Mills. 2009. Interleukin-1 and IL-23 induce innate IL-17 production from gammadelta T cells, amplifying Th17 responses and autoimmunity. *Immunity* 31: 331–341.
6. Colonna, M. 2009. Interleukin-22-producing natural killer cells and lymphoid tissue inducer-like cells in mucosal immunity. *Immunity* 31: 15–23.
7. Nograles, K. E., L. C. Zaba, A. Shemer, J. Fuentes-Duculan, I. Cardinale, T. Kikuchi, M. Ramon, R. Bergman, J. G. Krueger, and E. Guttman-Yassky. 2009. IL-22-producing "T22" T cells account for upregulated IL-22 in atopic dermatitis despite reduced IL-17-producing TH17 T cells. *J. Allergy Clin. Immunol.* 123: 1244–1252.
8. Duhen, T., R. Geiger, D. Jarrossay, A. Lanzavecchia, and F. Sallusto. 2009. Production of interleukin 22 but not interleukin 17 by a subset of human skin-homing memory T cells. *Nat. Immunol.* 10: 857–863.
9. Trifari, S., C. D. Kaplan, E. H. Tran, N. K. Crellin, and H. Spits. 2009. Identification of a human helper T cell population that has abundant production of interleukin 22 and is distinct from T(H)-17, T(H)1 and T(H)2 cells. *Nat. Immunol.* 10: 864–871.
10. Eyerich, S., K. Eyerich, D. Pennino, T. Carbone, F. Nasorri, S. Pallotta, F. Cianfarani, T. Odorisio, C. Traidl-Hoffmann, H. Behrendt, et al. 2009. Th22 cells represent a distinct human T cell subset involved in epidermal immunity and remodeling. *J. Clin. Invest.* 119: 3573–3585.
11. Xie, M. H., S. Aggarwal, W. H. Ho, J. Foster, Z. Zhang, J. Stinson, W. I. Wood, A. D. Goddard, and A. L. Gurney. 2000. Interleukin (IL)-22, a novel human cytokine that signals through the interferon receptor-related proteins CRF2-4 and IL-22R. *J. Biol. Chem.* 275: 31335–31339.
12. Kotenko, S. V., L. S. Izotova, O. V. Mirochnitchenko, E. Esterova, H. Dickensheets, R. P. Donnelly, and S. Pestka. 2001. Identification of the functional interleukin-22 (IL-22) receptor complex: the IL-10R2 chain (IL-10Rbeta) is a common chain of both the IL-10 and IL-22 (IL-10-related T cell-derived inducible factor, IL-TIF) receptor complexes. *J. Biol. Chem.* 276: 2725–2732.
13. Logsdon, N. J., B. C. Jones, K. Josephson, J. Cook, and M. R. Walter. 2002. Comparison of interleukin-22 and interleukin-10 soluble receptor complexes. *J. Interferon Cytokine Res.* 22: 1099–1112.
14. Li, J., K. N. Tomkinson, X. Y. Tan, P. Wu, G. Yan, V. Spaulding, B. Deng, B. Annis-Freeman, K. Heveron, R. Zollner, et al. 2004. Temporal associations between interleukin 22 and the extracellular domains of IL-22R and IL-10R2. *Int. Immunopharmacol.* 4: 693–708.
15. Renauld, J.-C. 2003. Class II cytokine receptors and their ligands: key antiviral and inflammatory modulators. *Nat. Rev. Immunol.* 3: 667–676.
16. Wolk, K., E. Witte, U. Reineke, K. Witte, M. Friedrich, W. Sterry, K. Asadullah, H. D. Volk, and R. Sabat. 2005. Is there an interaction between interleukin-10 and interleukin-22? *Genes Immun.* 6: 8–18.
17. Wolk, K., S. Kunz, E. Witte, M. Friedrich, K. Asadullah, and R. Sabat. 2004. IL-22 increases the innate immunity of tissues. *Immunity* 21: 241–254.
18. Aujla, S. J., Y. R. Chan, M. Zheng, M. Fei, D. J. Askew, D. A. Pociask, T. A. Reinhart, F. McAllister, J. Edeal, K. Gaus, et al. 2008. IL-22 mediates mucosal host defense against Gram-negative bacterial pneumonia. *Nat. Med.* 14: 275–281.
19. Zheng, Y., P. A. Valdez, D. M. Danilenko, Y. Hu, S. M. Sa, Q. Gong, A. R. Abbas, Z. Modrusan, N. Ghilardi, F. J. de Sauvage, and W. Ouyang. 2008. Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens. *Nat. Med.* 14: 282–289.
20. Boniface, K., F. X. Bernard, M. Garcia, A. L. Gurney, J. C. Lecron, and F. Morel. 2005. IL-22 inhibits epidermal differentiation and induces proinflammatory gene expression and migration of human keratinocytes. *J. Immunol.* 174: 3695–3702.
21. Ma, H. L., S. Liang, J. Li, L. Napierata, T. Brown, S. Benoit, M. Senices, D. Gill, K. Dunussi-Joannopoulos, M. Collins, et al. 2008. IL-22 is required for Th17 cell-mediated pathology in a mouse model of psoriasis-like skin inflammation. *J. Clin. Invest.* 118: 597–607.
22. Geboes, L., L. Dumoutier, H. Kelchtermans, E. Schurgers, T. Mitera, J.-C. Renauld, and P. Matthys. 2009. Proinflammatory role of the Th17 cytokine interleukin-22 in collagen-induced arthritis in C57BL/6 mice. *Arthritis Rheum.* 60: 390–395.
23. Radaeva, S., R. Sun, H. N. Pan, F. Hong, and B. Gao. 2004. Interleukin 22 (IL-22) plays a protective role in T cell-mediated murine hepatitis: IL-22 is a survival factor for hepatocytes via STAT3 activation. *Hepatology* 39: 1332–1342.
24. Sugimoto, K., A. Ogawa, E. Mizoguchi, Y. Shimomura, A. Andoh, A. K. Bhan, R. S. Blumberg, R. J. Xavier, and A. Mizoguchi. 2008. IL-22 ameliorates intestinal inflammation in a mouse model of ulcerative colitis. *J. Clin. Invest.* 118: 534–544.
25. Zenewicz, L. A., G. D. Yancopoulos, D. M. Valenzuela, A. J. Murphy, S. Stevens, and R. A. Flavell. 2008. Innate and adaptive interleukin-22 protects mice from inflammatory bowel disease. *Immunity* 29: 947–957.
26. Zenewicz, L. A., G. D. Yancopoulos, D. M. Valenzuela, A. J. Murphy, M. Karow, and R. A. Flavell. 2007. Interleukin-22 but not interleukin-17 provides protection to hepatocytes during acute liver inflammation. *Immunity* 27: 647–659.
27. Wolk, K., E. Witte, E. Wallace, W. D. Döcke, S. Kunz, K. Asadullah, H. D. Volk, W. Sterry, and R. Sabat. 2006. IL-22 regulates the expression of genes responsible for antimicrobial defense, cellular differentiation, and mobility in keratinocytes: a potential role in psoriasis. *Eur. J. Immunol.* 36: 1309–1323.
28. Baumann, H., and J. Gauldie. 1994. The acute phase response [see comment]. *Immunol. Today* 15: 74–80.
29. Gabay, C., and I. Kushner. 1999. Acute-phase proteins and other systemic responses to inflammation. [Published erratum appears in 1999 *N. Engl. J. Med.* 340: 1376.] *N. Engl. J. Med.* 340: 448–454.
30. Steel, D. M., and A. S. Whitehead. 1994. The major acute phase reactants: C-reactive protein, serum amyloid P component and serum amyloid A protein. *Immunol. Today* 15: 81–88.
31. Dumoutier, L., E. Van Roost, D. Colau, and J. C. Renauld. 2000. Human interleukin-10-related T cell-derived inducible factor: molecular cloning and functional characterization as an hepatocyte-stimulating factor. *Proc. Natl. Acad. Sci. USA* 97: 10144–10149.
32. Kozarsky, K. F., K. Jooss, M. Donahee, J. F. Strauss, III, and J. M. Wilson. 1996. Effective treatment of familial hypercholesterolaemia in the mouse model using adenovirus-mediated transfer of the VLDL receptor gene. *Nat. Genet.* 13: 54–62.
33. Bertone, A. L., D. D. Pittman, M. L. Bouxsein, J. Li, B. Clancy, and H. J. Seeherman. 2004. Adenoviral-mediated transfer of human BMP-6 gene accelerates healing in a rabbit ulnar osteotomy model. *J. Orthop. Res.* 22: 1261–1270.
34. Liang, S. C., A. J. Long, F. Bennett, M. J. Whitters, R. Karim, M. Collins, S. J. Goldman, K. Dunussi-Joannopoulos, C. M. Williams, J. F. Wright, and L. A. Fouser. 2007. An IL-17F/A heterodimer protein is produced by mouse Th17 cells and induces airway neutrophil recruitment. *J. Immunol.* 179: 7791–7799.
35. Ki, S. H., O. Park, M. Zheng, O. Morales-Ibanez, J. K. Kolls, R. Bataller, and B. Gao. 2010. Interleukin-22 treatment ameliorates alcoholic liver injury in a murine model of chronic-binge ethanol feeding: Role of STAT3. *Hepatology.* DOI: 10.1002/hep.23837.
36. Margarson, M. P., and N. Soni. 1998. Serum albumin: touchstone or totem? *Anaesthesia* 53: 789–803.
37. Yeh, S.-S., K. Blackwood, and M. W. Schuster. 2008. The cytokine basis of cachexia and its treatment: are they ready for prime time? *J. Am. Med. Dir. Assoc.* 9: 219–236.
38. Li, Q., M. A. Kay, M. Finegold, L. D. Stratford-Perricaudet, and S. L. Woo. 1993. Assessment of recombinant adenoviral vectors for hepatic gene therapy. *Hum. Gene Ther.* 4: 403–409.
39. Brand, S., J. Dambacher, F. Beigel, K. Zitzmann, M. H. Heeg, T. S. Weiss, T. Prüfer, T. Olszak, C. J. Steib, M. Storr, et al. 2007. IL-22-mediated liver cell regeneration is abrogated by SOCS-1/3 overexpression in vitro. *Am. J. Physiol. Gastrointest. Liver Physiol.* 292: G1019–G1028.
40. Ren, X., and L. M. Colletti. 2010. IL-22 is involved in liver regeneration after hepatectomy. *Am. J. Physiol. Gastrointest. Liver Physiol.* 298: G74–G80.
41. Gruver, A. L., and G. D. Sempowski. 2008. Cytokines, leptin, and stress-induced thymic atrophy. *J. Leukoc. Biol.* 84: 915–923.
42. Carson, F. 1997. Nuclear and cytoplasmic staining. In: *Histotechnology: A Self-Instructional Text*, 2nd ed. F. L. Carson, ed. American Society for Clinical Pathology, Chicago, IL, p. 83–109.
43. Seely, J. 1999. Kidney. In *Pathology of the Mouse.* R. R. Maronpot, B. W. Gaul, and G. A. Boorman, eds. Cache River Press, Vienna, IL, p. 207–234.
44. Greaves, P. 2007. Urinary tract. In *Histopathology of Preclinical Toxicity Studies*, 3rd ed. P. Greaves, ed. Elsevier Science, New York, p. 570–660.
45. Pan, H., F. Hong, S. Radaeva, and B. Gao. 2004. Hydrodynamic gene delivery of interleukin-22 protects the mouse liver from concanavalin A-, carbon tetrachloride-, and Fas ligand-induced injury via activation of STAT3. *Cell. Mol. Immunol.* 1: 43–49.

46. Dambacher, J., F. Beigel, K. Zitzmann, M. H. Heeg, B. Göke, H. M. Diepolder, C. J. Auernhammer, and S. Brand. 2008. The role of interleukin-22 in hepatitis C virus infection. *Cytokine* 41: 209–216.
47. Uhlar, C. M., and A. S. Whitehead. 1999. Serum amyloid A, the major vertebrate acute-phase reactant. *Eur. J. Biochem.* 265: 501–523.
48. Su, S. B., W. Gong, J. L. Gao, W. Shen, P. M. Murphy, J. J. Oppenheim, and J. M. Wang. 1999. A seven-transmembrane, G protein-coupled receptor, FPRL1, mediates the chemotactic activity of serum amyloid A for human phagocytic cells. *J. Exp. Med.* 189: 395–402.
49. Cheng, N., R. He, J. Tian, P. P. Ye, and R. D. Ye. 2008. Cutting edge: TLR2 is a functional receptor for acute-phase serum amyloid A. *J. Immunol.* 181: 22–26.
50. Missé, D., H. Yssel, D. Trabattoni, C. Oblet, S. Lo Caputo, F. Mazzotta, J. Pène, J. P. Gonzalez, M. Clerici, and F. Veas. 2007. IL-22 participates in an innate anti-HIV-1 host-resistance network through acute-phase protein induction. *J. Immunol.* 178: 407–415.
51. Ivanov, I. I., K. Atarashi, N. Manel, E. L. Brodie, T. Shima, U. Karaoz, D. Wei, K. C. Goldfarb, C. A. Santee, S. V. Lynch, et al. 2009. Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell* 139: 485–498.
52. Kelly, K. J., B. Kluve-Beckerman, and J. H. Dominguez. 2009. Acute-phase response protein serum amyloid A stimulates renal tubule formation: studies in vitro and in vivo. *Am. J. Physiol. Renal Physiol.* 296: F1355–F1363.