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Recurrent Staphylococcal Cellulitis and Subcutaneous Abscesses in a Child with Autoantibodies against IL-6

Anne Puel, Capucine Picard, Mathie Lorrot, Charlotte Pons, Maya Chrabieh, Lazaro Lorenzo, Maria Mamani-Matsuda, Emmanuelle Jouanguy, Dominique Gendrel, and Jean-Laurent Casanova

We investigated an otherwise healthy patient presenting two episodes of staphylococcal cellulitis and abscesses, accompanied by high fever and biological signs of inflammation but, paradoxically, with no detectable increase in serum levels of C-reactive protein (CRP), an IL-6-responsive protein synthesized in the liver. Following in vitro activation of whole blood cells from the patient with multiple cytokines, TLR agonists, heat-killed bacteria, and mitogens, we observed a profound and specific impairment of IL-6 secretion. However, the patient’s PBMCs, activated in the same conditions but in the absence of the patient’s plasma, secreted IL-6 normally. The patient’s serum contained high titers of IgG1 autoantibodies against IL-6, which specifically neutralized IL-6 production by control PBMCs as well as IL-6 responses in the human hepatocellular carcinoma cell line Hep3B. These anti-IL-6 autoantibodies were detected over a period of 4 years, in the absence of any other autoantibodies. Our results indicate that these Abs probably prevented an increase in CRP concentration during infection and that impaired IL-6-mediated immunity may have contributed to staphylococcal disease. Patients with severe bacterial infections and low serum CRP concentrations should be tested for anti-IL-6 autoantibodies, especially in the presence of other clinical and biological signs of inflammation. The Journal of Immunology, 2008, 180: 647–654.

Increased susceptibility to staphylococcal diseases is a hallmark of several primary immunodeficiencies, including chronic granulomatous disease (1), autosomal dominant hyper-IgE syndrome (2), anhidrotic ectodermal dysplasia with immunodeficiency, and IL-1R-associated kinase (IRAK)-4 deficiency (reviewed in Refs. 3 and 4). In the last three of these conditions, infectious episodes are typically associated with mild or delayed clinical and biological signs of inflammation, such as fever and an increase in erythrocyte sedimentation rate, serum fibrinogen, and C-reactive protein (CRP) levels. Procalcitonin (PCT) levels have not been studied in these patients (3, 4). The molecular basis of the autosomal dominant hyper-IgE syndrome was recently found to result from mutations in STAT3, impairing in particular IL-6 and IL-10 responses (5). These data were soon confirmed by other investigators (6). IRAK-4 deficiency results in impaired signaling downstream from TLRs and IL-1 receptors, which belong to the Toll-IL-1 receptor superfamily. Anhidrotic ectodermal dysplasia with immunodeficiency patients bearing NEMO or IKBA mutations show impaired signaling downstream from receptors of the TIR and TNF receptor superfamilies. Mutations in STAT3, IRAK4, NEMO, or IKBA result in the impaired production of and/or response to multiple proinflammatory cytokines, such as IL-6, TNF-α, IL-1β, or IL-12, by the patients’ cells in response to various stimuli. However, the relationship between these immunological abnormalities and the occurrence of staphylococcal disease is unclear.

We studied a boy with recurrent staphylococcal cellulitis and s.c. abscesses. He showed clinical and biological signs of inflammation but had no detectable CRP in his serum during infections. CRP is synthesized in the liver, under the tight control of IL-6 (Refs. 7–16; reviewed in Ref. 17). We therefore thought that the pathogenesis of staphylococcal disease in this patient might provide us with an opportunity to explore the mechanisms governing antistaphylococcal immunity. In marked contrast to what has been reported for patients with mutations in NEMO, IKBA, or IRAK4, the in vitro stimulation of whole blood cells from this patient resulted in the normal production of all cytokines tested, with the exception of IL-6, regardless of the stimulus used, including mi- togens. However, the patient’s PBMCs, isolated from his serum, secreted IL-6 when activated in the same conditions. We found high levels of circulating, neutralizing IgG1 autoantibodies directed against IL-6 in the patient’s plasma, accounting for his lack of CRP response and, possibly, for the development of staphylococcal disease. This is the first report of an association between an autoantibody against IL-6 and a clinical infectious disease.

Materials and Methods

Case reports

A boy born to nonconsanguineous parents from Haiti, living in France, was hospitalized at the age of 11 months after he developed chickenpox complicated with multiple cellulitis lesions and s.c. abscesses caused by...
Staphylococcus aureus on his face, chest, back, right hip, and left and right upper legs. He was treated with i.v. acyclovir, parenteral antibiotics, and surgical draining. No CRP was detected in serum during this infection (<5 mg/L). The patient had high fever (39°C), high neutrophil counts (11,300/mm³), and high serum PCT levels (2 ng/ml; normal values, ≤0.5 ng/ml). When bitten by mosquitoes in Haiti at the age of 2 years and 5 months, the patient had staphylococcal folliculitis and cellulitis on the face and chest. He responded well to oral antibiotic treatment. Since these two episodes of unusually severe cutaneous staphylococcal disease, the patient has been doing well without prophylactic treatment.

Immunological work-up at 17 mo of age showed normal numbers of T and B lymphocytes, and normal proportions of the CD4+, CD8+, CD4+, CD8+, and CD19+ cells, with 2550 lymphocytes/mm³. The patient had mononuclear (850 neutrophils/mm³), Ig levels were also within normal ranges for age: IgG, 7.17 mg/ml (normal range, 3.35–6.23); IgA, 0.26 mg/ml (0.27–1.06); and IgM 1.07 mg/ml (0.48–1.43). At 21 mo of age, allomagglutinin titers were 1/32. Serum Abs against tetanus toxoid (0.27–1.06 mg/ml) and IgM or IgG against varicella-zoster virus (VZV) were detected. No Abs were detected against human T cell lymphotropic virus types I and II. At the age of 4 years, the patient had no IgM or IgG against human HSV-1, -2, and CMV, no IgM against EBV or its viral capsid Ag or IgG against EBV-encoded nuclear Ag. The patient had positive titers of IgG against viral capsid Ag (124 U/ml; positive, >20 U/ml) and IgG against VZV (358 U; negative, <90 U).

Plasma and PBMCs

Plasma samples were obtained from the patient studied, from 12 other patients (half male and half female, ranging from 2 to 15 years of age), with various forms of severe staphylococcal diseases (10 had cellulitis and s.c. abscesses, 4 had pneumonia, 2 had septicaemia, and 2 had meningitis), 10 patients with recurrent or severe chickenpox infections (male half and male female, ranging from 1 to 20 years; 4 had encephalitis, 2 had recurrent infections, 1 had cellulitis, and 1 had systemic vasculitis) and from 25 healthy controls (about half male and half female, from 24 to 50 years of age), by collecting blood into heparin-coated tubes, centrifuging, and freezing the supernatants at −20°C. PBMCs were prepared by density gradient centrifugation, using Ficoll-Paque Plus (Amersham Biosciences) and were cultured in RPMI 1640 supplemented with 10% heat-inactivated FBS (In vitrogen Life Technologies) or 10% human plasma.

Cell activation

Whole blood samples were diluted 1/2 in RPMI 1640 (Invitrogen Life Technologies) and activated with IL-1β (20 ng/ml; R&D Systems), TNF-α (20 ng/ml; R&D Systems), LPS (from Salmonella minnesota R595, 10 μg/ml; Sigma-Aldrich), heat-killed S. aureus (5 × 10⁶ particles/ml; American Type Culture Collection; LGC Promochem), PMA plus ionomycin (10⁻⁷ M and 10⁻⁸ M, respectively; Sigma-Aldrich) or PHA-P (diluted 1/100; Bacto PHA-P, BD Biosciences).

Supernatants were collected after 24 and 48 h of stimulation. For PBMCs, 2 × 10⁶ cells/well/ml were activated in the same conditions as whole blood, and supernatants were harvested after 48 h. SV40-transformed fibroblasts from the patient or controls were cultured at a density of 3 × 10⁶ cells/well in six-well plates, in DMEM (Invitrogen Life Technologies) supplemented with 10% FBS and stimulated with TNF-α (20 ng/ml), IL-1β (20 ng/ml), or PMA (10⁻⁷ M) plus ionomycin (10⁻⁶ M). Supernatants were collected after 48 h at activation. After centrifugation, the supernatant (200 μl) was added to 96-well plates coated with capture mAbs against human IL-6, IL-8, IL-10, IL-12p70, IL-13, IL-17, G-CSF, GM-CSF, IFN-γ, TNF-α, MCP-1, and MIP-1β (Bio-Rad), according to the manufacturer’s instructions. Cytokine production in whole-blood supernatants was normalized according to the number of PBMCs in the individuals tested. IL-6-soluble receptor concentrations were determined with the human IL-6-soluble receptor ELISA immunoassay kit (R&D Systems), according to the manufacturer’s instructions. CRP production was evaluated using Hep3B cells, left unstimulated or stimulated with 50 ng/ml IL-6 for 4–36 h, cultured with 10% plasma from the patient or a control, according to the manufacturer’s instructions (Quanti- tiek; R&D Systems).

Detection of anti-IL-6 Abs by ELISA

Maxisorp 96-well ELISA plates (Maxisorp; Nunc) were coated by incubation overnight at 4°C with 2 μg/ml rIL-6 (R&D Systems). They were then blocked (PBS-Tween 0.05%), blocked by incubation with the same buffer supplemented with 5% nonfat milk powder, washed, and incubated with serial dilutions of plasma samples from the patient or from controls for 2 h at room temperature. Plates were thoroughly washed. Fc-specific HRP-conjugated IgG fractions of polyclonal goat antiserum against human IgG were added to a final concentration of 2 μg/ml. Plates were incubated for 1 h at room temperature and washed. Substrate was added, and optical density was measured. The anti-IL6 IgG subclass was determined, by adding Abs against human IgG1 (dilution, 1/4000), IgG2 (1/2700), IgG3 (1/8700), and IgG4 (1/8700) to IL-6-coated plates previously incubated with serial dilutions of serum samples from the patient and controls for 18 h at 4°C. The plates were washed and incubated for 1 h at room temperature with peroxidase-conjugated anti-IgG Ab diluted 1/15,000 in PBS-Tween, 0.05%.

Western blotting

We subjected 500 ng of rIL-6 (R&D Systems) or BSA to SDS-PAGE (10% acrylamide) under reducing conditions and electroblotted the resulting bands onto nitrocellulose membranes (Schleicher & Schuell). Membranes were blocked by incubation for 1 h at room temperature with PBS supplemented with 5% BSA and 0.05% Tween 20, washed, and incubated overnight at 4°C with plasma samples from the patient or controls, diluted 1/100 in PBS, 5% BSA, 0.01% Tween 20. The membranes were washed three times and incubated for 1 h at room temperature with HRP-coupled anti-human IgG, used at a final concentration of 2 μg/ml. The membranes were washed three times and developed for ECL (Amersharm).

We evaluated Stat3 phosphorylation in Hep3B cells by plating 4 × 10⁵ cells in 60-mm tissue culture dishes (BD Biosciences), in 5 ml of MEM containing essential amino acids and sodium pyruvate supplemented with 10% plasma from the patient or controls, and incubating them overnight at 37°C in an atmosphere containing 5% CO₂. The cells were left unstimulated or stimulated with 50 ng/ml IL-6 for 60 min. Total cell extracts were prepared using a lysis buffer containing 50 mM Tris-HCl pH 7.5, 150 mM NaCl, 2 mM EDTA, 0.5% Triton X-100 and protease and phosphatase inhibitors. Proteins (50 μg) were separated by reducing SDS-PAGE and electrophoresed onto nitrocellulose membranes (Schleicher & Schuell), STAT-3 phosphorylation and GAPDH expression were evaluated using anti-human phospho-STAT-3 (Tyr705) polyclonal rabbit Abs (Cell Signaling no. 9131) and anti-human GAPDH polyclonal rabbit Abs (Santa Cruz Biotechnology; sc-25778), according to the manufacturer’s instructions.

IgG depletion

Plasma from the patient was subjected to standard IgG affinity chromatography purification, using protein G beads (Sigma-Aldrich), according to the manufacturer’s instructions. Briefly, we incubated 300 μl of plasma from the patient for 1 h with 1 μl of protein G beads, with shaking. Protein G-bound IgG fractions were then eluted in 0.1 M glycine, pH 2.7, and neutralized to pH 7.4 with Tris, pH 9 (flowthrough fractions). Samples taken before and after protein G binding and the five first recovered fractions that had bound to the protein G (flowthrough fractions), were all subjected to anti-IL-6 ELISA.

Flow cytometry analysis

For the intracellular staining of IL-6 and TNF-α, 2 × 10⁶ PBMCs/ml were left unstimulated or were stimulated for 2, 4, 7, or 24 h with LPS (10 μg/ml) in the presence of brefeldin A (10 μg/ml). They were then fixed and permeabilized with the IntraStain kit (DakoCytomation). PBMCs were first incubated with anti-human CD14-FITC or with an isotype control (BD Pharmingen). They were then washed and incubated with anti-human IL-6-PE, anti-human TNF-α-PE, or an isotype control-PE. Cells were washed and analyzed by flow cytometry on a FACScan (BD Biosciences).
Molecular genetics

Total RNA was isolated from PBMCs using Trizol (Invitrogen Life Technologies). The full-length IL-6 cDNA, including the 5′- and 3′-untranslated regions (UTR), the full-length CRP cDNA, and the GAPDH cDNA were amplified with specific primers (primers and conditions for PCR amplification and sequencing are available upon request). The full-length IL-6 cDNA and the 5′- and 3′-UTR regions were sequenced using the Big Dye Terminator cycle sequencing kit (PE France; Applied Biosystems) and analyzed on an ABI Prism 3700 apparatus (Applied Biosystems).

Results

Lack of detectable IL-6 secretion by whole blood cells from the patient

On the basis of the patient’s clinical and biological phenotype, we initially suspected that he had a subtle defect in NF-κB-mediated immunity (3). We therefore stimulated whole blood cells from the patient with IL-1β, LPS, TNF-α, and heat-killed S. aureus and with the mitogens PMA-ionomycin and PHA, serving as positive controls of cell activation. The stimulation of whole blood cells from healthy controls resulted in the production of large amounts of IL-6, whereas very low levels of IL-6 were reproducibly detected with the patient’s cells, in all stimulation conditions, after 24 or 48 h (data not shown; Fig. 1A). Whole blood from >800 individuals (ranging from 3 mo to 60 years) has been tested for IL-6 production in the same conditions of activation. Although, as shown in Fig. 1A, there is some variability in IL-6 production, none of them displayed a phenotype comparable with that of the patient, i.e., the lack of detectable IL-6 in the supernatant (data not shown). In contrast, the production of other cytokines, interleukins, IFNs, chemokines, and growth factors, including IL-1β, IL-2, IL-4, IL-5, IL-7, IL-8, IL-10, IL-12p40, IL-12p70, IL-13, IL-17, G-CSF, GM-CSF, IFN-γ, TNF-α, MCP-1, and MIP-1β, was comparable with the controls tested (Fig. 1B–D, and data not shown). The patient thus appeared to have a profound, but apparently specific defect in IL-6 production and secretion into the extracellular medium. However, no mutation was found in the full-length IL-6 cDNA, including the 5′- and 3′-UTR regions (data not shown).

Normal induction of IL-6 by the patient’s lymphocytes

We tried to discriminate between defects of IL-6 synthesis and secretion by carrying out intracellular staining for IL-6, with TNF-α used as the positive control. Whole blood cells and PBMCs from the patient and controls were left unstimulated or were stimulated with LPS. Three to 24 h after stimulation with LPS, both cytokines were detected, in similar amounts, in CD14+ monocytes from the patient and from controls (Fig. 2A, and data not shown). Consistent with these findings, IL6 mRNA induction was normal in PBMCs from the patient after stimulation with IL-1β and LPS for 2–12 h, as determined by semiquantitative RT-PCR, using the housekeeping gene encoding GAPDH as a control (data not shown). Thus, the patient’s monocytes were intrinsically able to synthesize IL-6 mRNA and protein, which was not, however, detectable in the cell supernatant. Moreover, PBMCs from the patient, cultured with FBS in the absence of autologous plasma and stimulated with IL-1β, LPS, PHA, or PMA-ionomycin, secreted IL-6 at normal levels (Fig. 2B). Normal IL-6 secretion was also observed when SV40-transformed fibroblasts from the patient were stimulated with IL-1β, TNF-α, or PMA-ionomycin, or when EBV-transformed B cells from the patient were stimulated with phorbol diester butyrate (data not shown). These results suggest that patient’s cells produced and secreted IL-6 normally and that the patient’s plasma contained a soluble factor preventing IL-6 detection.

An IL-6-neutralizing factor in the patient’s serum

We tested this hypothesis by incubating control PBMCs with a medium containing 10% plasma from the patient or various controls, leaving these cells unstimulated or stimulating them with LPS for 48 h and then determining IL-6 and TNF-α concentrations in the supernatants. Large amounts of IL-6 were detected in the supernatants of activated PBMCs in the presence of control plasma, whereas IL-6 was barely detectable in the presence of serum from the patient (Fig. 3A, left). In marked contrast, levels of TNF-α production by control PBMCs were similar in the presence of both types of plasma (Fig. 3A, right). Moreover, when various doses of rIL-6 (rIL-6) were incubated for 30 min with 10% of plasma from the patient and free rIL-6 levels were then determined by classical ELISA, almost no rIL-6 was detected, whereas rIL-6 was readily detected when the initial incubation was with control plasma. At the highest dose (50 ng of rIL-6), only 6.5 ng of rIL-6 were detected following incubation with the patient’s plasma (Fig. 3B). These results provide evidence for the existence of a soluble
factor in the patient’s serum that strongly inhibited the detection of IL-6.

Plasma autoantibodies against IL-6

Similar levels of soluble IL-6R α-chain were found in the plasma or blood supernatants from controls and the patient, following stimulation with LPS (data not shown and Fig. 4A). However, when we assessed the levels of autoantibodies against IL-6 in the patient’s serum, high IgG titers were identified, whereas controls’ sera displayed almost no anti-IL-6 activity (Fig. 4B). To test the hypothesis that these Abs might result from molecular mimicry with *S. aureus* or from immune deregulation after severe VZV infection, we investigated the presence of autoantibodies against IL-6 in the patient’s serum. We found high levels of IgG anti-IL-6 Abs in the patient’s serum, whereas controls’ sera displayed almost no anti-IL-6 activity (Fig. 4B).

**FIGURE 2.** A, IL-6 protein induction in PBMCs from controls and the patient upon stimulation. IL-6 and TNF-α protein induction (in green) in PBMCs from a control and the patient upon 4 h of stimulation with LPS (10 μg/ml) measured by flow cytometry. Intracellular staining was evaluated in CD14+ gated cells. This experiment is representative of two independent experiments. B, IL-6 production in the culture supernatants of PBMCs from the patient and controls cultured in RPMI 10% FBS, measured by ELISA, in response to 48 h of stimulation with IL-1β (20 ng/ml), LPS (10 μg/ml), PHA (1/700), and PMA-ionomycin (Io; 10⁻⁷ M and 10⁻⁸ M, respectively). Means and SDs correspond to three different controls, and the patient tested on two different occasions.

**FIGURE 3.** A, IL-6 and TNF-α production in the supernatants of control PBMCs cultured in RPMI supplemented with 10% human control serum or serum from the patient, in response to 48 h of LPS (10 μg/ml) activation. Means and SDs correspond to three plasma samples from three different controls and two plasma samples from the patient taken at two different time points. B, rIL-6 determination by ELISA. Various fixed doses of rIL-6 were first incubated in the presence of 10% plasma from a control or the patient, diluted in RPMI 1640, for 30 min. The supernatants were then subjected to an anti-IL-6 ELISA to determine the remaining unbound rIL-6.
infection, we measured anti-IL-6 autoantibody levels in 12 patients with severe staphylococcal diseases and 10 patients with severe chickenpox. None of these patients had any anti-IL-6 autoantibodies comparable with those found in our patient (Fig. 4C). The patient’s Abs could be removed by passing the serum through a protein G-Sepharose column, with the recovered flowthrough fractions that had bound to the protein G displaying anti-IL-6 activity (Fig. 4D). When rIL-6 and BSA were subjected to SDS-PAGE and the resulting membrane was incubated in the presence of plasma from the patient or controls diluted 1/100, a strong band corresponding to rIL-6 was detected with the patient’s plasma only (Fig. 4E). The patient therefore had high titers of neutralizing circulating IgG autoantibodies against IL-6. Finally, using mouse Abs directed against the four IgG subclasses, we showed by ELISA that these IgG autoantibodies were mostly of the IgG1 subclass (Fig. 4F).

**Deficient IL-6 signaling in Hep3B cells cultured in the presence of the patient’s plasma**

Finally, we used the human hepatocellular carcinoma cell line Hep3B to determine whether the anti-IL-6 autoantibodies found in the patient’s plasma were responsible for the lack of increase in CRP levels during staphylococcal infections. Almost no Stat3 phosphorylation was detected upon IL-6 stimulation in Hep3B initially incubated with medium containing 10% plasma from the patient, whereas phosphorylation was observed when the cells were incubated with control plasma (Fig. 5A). Similarly, Hep3B cells activated with IL-6 for various periods of time (from 4 to 36 h) showed almost no induction of CRP mRNA (Fig. 5B) or protein production (Fig. 5C) when incubated with the plasma from the patient, whereas induction was observed with control plasma. Overall, the data obtained with this hepatocyte cell line in vitro strongly suggested that IL-6 responses in hepatocytes in vivo were impaired by the presence of high levels of IgG1 autoantibodies against IL-6. These

**FIGURE 4.** A, Measurement of the soluble IL-6R α-chain by ELISA, in whole blood culture supernatants from controls and the patient at 48 h, with no stimulation or upon stimulation with 10 μg/ml LPS. Means and SDs correspond to four different controls and results obtained for the patient at four different times. B, Anti-IL-6 IgG titers measured by ELISA in 5 different plasma samples from the patient (taken at different time periods) and in 15 different healthy controls. OD_{630-490} is plotted against serum dilution. C, Anti-IL-6 IgG titers measured by ELISA in five different plasma samples from the patient, in plasma samples from 12 patients presenting severe staphylococcal diseases (S. aureus), and from 10 patients presenting severe chickenpox (VZV). D, Anti-IL-6 IgG titers measured by ELISA in three different plasma samples from the patient before and after IgG depletion by protein G-Sepharose and in the first five recovered flowthrough fractions bound to protein G. E, IL-6 Western blot using plasma from the patient and controls, diluted 1/100, incubated with rIL-6 500 or BSA ng previously transferred to polyvinyl membrane. The band at ~20 kDa corresponds to rIL-6. This experiment is representative of two independent experiments. F, Anti-IL-6 AutoAb isotype determination by ELISA. Anti-IL-6 IgG subclasses in plasma samples from a control and from the patient. High IgG1 and low IgG3 levels were detected only in the patient’s plasma. OD_{630-490} is plotted against serum dilution.

**FIGURE 5.** A, Measurement of Stat3 phosphorylation, by Western blot, in Hep3B cells upon IL-6 stimulation in the presence of 10% plasma from the patient or controls. B and C, CRP mRNA and protein induction, at 36 h, in Hep3B cells, following IL-6 stimulation in the presence of 10% plasma from the patient or controls.
autoantibodies are therefore probably responsible for the lack of increase in CRP concentration in the patient during infections.

**Discussion**

We investigated an otherwise healthy child with two episodes of severe cutaneous staphylococcal disease, including cellulitis and s.c. abscesses requiring surgical drainage, occurring at the ages of 11 and 29 mo. These two episodes were associated with undetectable levels of CRP in serum, despite the presence of other clinical and biological signs of inflammation. The child’s blood cells displayed a profound impairment in IL-6 secretion ex vivo and, presumably, in vivo, because of the presence of circulating high-affinity IL-6-specific IgG1 autoantibodies. These autoantibodies were identified when the patient was 18 mo old and were documented over a period of 4 years. No other autoantibodies directed against blood cells, tissues (kidney, liver, and stomach), DNA, nuclear, or cytoplasmic proteins were detected in the patient’s plasma. Apart from his staphylococcal disease, the patient had no other unusually severe infectious disease and, has been doing well since his last infectious episode 3 years ago. The presence of anti-IL-6 autoantibodies most likely accounts for the lack of increase in CRP concentration during staphylococcal disease, given that IL-6 has been unambiguously shown to play a key role in CRP induction (reviewed in Ref. 17). In addition, no CRP induction was observed in vitro by the stimulation of the hepatocyte cell line Hep3B with IL-6 following incubation with the patient’s plasma (Fig. 5, B and C). Conversely, the production of other proinflammatory cytokines, such as TNF-α, IL-1β, IL-12 and IL-8, upon whole blood or PBMC activation in vitro, reached levels comparable to that of controls’ cells (Figs. 1, B–D). This probably accounted for the otherwise normal inflammatory reaction seen in vivo, including cutaneous pus production, fever, and high PCT concentration. The selective IL-6 deficiency thus resulted in an apparently selective lack of increase in serum CRP concentration.

There are several possible explanations for the pathogenesis of anti-IL-6 autoantibodies in our patient. The presence of these autoantibodies is unlikely to result from an immune deregulation following the patient’s VZV or staphylococcal infection, whether by molecular mimicry or by other mechanisms. Indeed, the course of events in our patient is much more suggestive of the IL-6 autoantibodies preceding the VZV infection as well as the two episodes of staphylococcal disease. Moreover, none of the other patients tested presenting with severe and/or recurrent VZV or staphylococcal diseases showed such anti-IL-6 neutralizing IgG titers (Fig. 4C). A genetic disorder leading to the induction of impaired self-tolerance is more likely to be involved, as seen in the complex autoimmune polyendocrinopathy syndrome type I (APS1 or APECED), resulting from mutations in AIRE (18, 19). AIRE normally promotes the ectopic expression in the thymus of peripheral tissue-restricted Ags (20). A recent study showed a tight correlation between the APECED syndrome and the presence of very high titers of IgG-neutralizing autoantibodies against type I IFNs (21). Although not genetically ruled out, mutations in AIRE are unlikely in our patient, because he lacks all known features of APECED and neither IL-6-specific autoantibodies nor staphylococcal diseases were reported to our knowledge in APECED patients. Others hitherto unknown inborn immune disorders may, however, account for the selective appearance of anti-IL-6 autoantibodies in our patient (22).

The association of two rare events, IL-6 deficiency and staphylococcal disease, further suggests that there might be a causal relationship. IL-6 plays a major role in inflammation and acute-phase reactions (23), and mice lacking IL-6 have impaired inflammatory and acute-phase responses (24). To our knowledge, IL-6-deficient mice have not been challenged with *S. aureus* but are plausibly vulnerable, given that they are susceptible to a large number of other pyogenic bacteria, such as *Streptococcus pneumoniae* (25), *Streptococcus pyogenes* (26), *Klebsiella pneumoniae* (27), *Pseudomonas aeruginosa* (28), and *Escherichia coli* (29, 30). In addition, the up-regulation of IL-6 signaling by transient injections of soluble IL-6 receptor has been shown to increase mouse survival rates and *S. aureus* clearance (31). The existence of a relationship between IL-6 deficiency and susceptibility to *S. aureus* also recently gained credence from two human studies. First, tyrosine kinase 2 deficiency was found in a patient displaying in particular IL-6 and IL-10 hyporesponsiveness and staphylococcal disease (32). Second, mutations in STAT3 were shown to be responsible for an autosomal dominant form of the hyper-IgE syndrome (5, 6). A hallmark of this syndrome is recurrent staphylococcal skin abscesses with impaired CRP increase during infections, and the patients’ blood cells showed defective responses to IL-6 and IL-10 (5, 6). In TYK2- and STAT3-deficient patients, the impact of impaired responses to IL-6 may be involved in the pathogenesis of the staphylococcal disease seen in these patients. Other cytokines, such as IL-10, may be involved, however, and definitive causal relationship between IL-6 deficiency and staphylococcal disease will be established only with the identification of patients with IL-6 or IL-6R deficiency.

If so, the mechanisms by which IL-6 deficiency might predispose to infection are unclear. In particular, the role of CRP cannot be assessed in mice, in which it is present only as a trace serum protein and is not an acute-phase marker (33). However, mice transgenic for human CRP have been shown to be protected against death following infection with the Gram-positive bacterium *S. pneumoniae* (34–36), through direct binding to the phosphocholine residues present in the bacterial cell wall. CRP has also been shown to bind various other pathogens, including *Haemophilus influenzae* (37), *Neisseria* spp (38), *A. fumigatus* (39) and *C. albicans* (40), although CRP-mediated protection experiments have not yet been performed for all these pathogens. No such binding has been observed for *S. aureus* (41, 42), but mice transgenic for human CRP were also shown to be protected after infection with the Gram-negative pathogen *Salmonella typhimurium*, despite an absence of CRP binding to this pathogen (43). The potential protective effects of CRP remain unclear (44), but the absence of serum CRP in our patient may have played a role in his susceptibility to *S. aureus*.

Finally, our findings should be considered in the context of recent reports concluding that autoantibodies against IFN-γ confer a predisposition to severe mycobacterial diseases (45–49). However, not all autoantibodies are apparently pathogenic, as autoantibodies against various cytokines have been identified in healthy individuals, patients suffering from various immunoinflammatory diseases, and patients treated with the corresponding recombinant cytokines (50–74). In particular, natural anti-IL-6 autoantibodies have been found in plasma samples from apparently healthy individuals (75–81) and in patients with rheumatoid arthritis (82), systemic sclerosis (83, 84), or alcoholic liver cirrhosis (85). These anti-IL-6 autoantibodies have not been reported to be associated with an increase in susceptibility to bacterial infections. Two of the 25 healthy controls we tested had detectable anti-IL-6 Abs in their plasma, but, in marked contrast to our patient, their titers were much lower and had no neutralizing activity (data not shown). In addition, none of the 800 individuals activated and tested under the same conditions as those of our patient showed a defect of IL-6 production comparable with our patient, suggesting that the neutralizing activity of our patient’s Abs is a rare event, as previously reported (81). Therefore, our identification of a patient with high
titers of highly neutralizing autoantibodies against IL-6, together with a strong susceptibility to severe staphylococcal disease, could potentially extend the concept of autoantibodies against cytokines increasing susceptibility to pathogens. The fact that neutralizing autoantibodies against IL-6 have also been found in 0.1% of apparently healthy individuals (81) however suggests that either IL-6 deficiency has a pathogenic impact under certain conditions and/or that its clinical outcome can improve with age, as seen in our patient and in IRAK-4-deficient patients (86). AutoAbs against cytokines should therefore be sought in patients presenting unexplained severe infectious diseases. AutoAbs against IL-6 should be sought, in particular, if serum CRP concentration is specifically explained severe infectious diseases. AutoAbs against IL-6 titers of highly neutralizing autoantibodies against IL-6, together with a strong susceptibility to severe staphylococcal disease, could potentially extend the concept of autoantibodies against cytokines increasing susceptibility to pathogens. The fact that neutralizing autoantibodies against IL-6 have also been found in 0.1% of apparently healthy individuals (81) however suggests that either IL-6 deficiency has a pathogenic impact under certain conditions and/or that its clinical outcome can improve with age, as seen in our patient and in IRAK-4-deficient patients (86). AutoAbs against cytokines should therefore be sought in patients presenting unexplained severe infectious diseases. AutoAbs against IL-6 should be sought, in particular, if serum CRP concentration is specifically explained severe infectious diseases. AutoAbs against IL-6 titers of highly neutralizing autoantibodies against IL-6, together with a strong susceptibility to severe staphylococcal disease, could potentially extend the concept of autoantibodies against cytokines increasing susceptibility to pathogens. The fact that neutralizing autoantibodies against IL-6 have also been found in 0.1% of apparently healthy individuals (81) however suggests that either IL-6 deficiency has a pathogenic impact under certain conditions and/or that its clinical outcome can improve with age, as seen in our patient and in IRAK-4-deficient patients (86). AutoAbs against cytokines should therefore be sought in patients presenting unexplained severe infectious diseases. AutoAbs against IL-6 should be sought, in particular, if serum CRP concentration is specifically explained severe infectious diseases. AutoAbs against IL-6 titers of highly neutralizing autoantibodies against IL-6, together with a strong susceptibility to severe staphylococcal disease, could potentially extend the concept of autoantibodies against cytokines increasing susceptibility to pathogens. The fact that neutralizing autoantibodies against IL-6 have also been found in 0.1% of apparently healthy individuals (81) however suggests that either IL-6 deficiency has a pathogenic impact under certain conditions and/or that its clinical outcome can improve with age, as seen in our patient and in IRAK-4-deficient patients (86). AutoAbs against cytokines should therefore be sought in patients presenting unexplained severe infectious diseases. AutoAbs against IL-6 should be sought, in particular, if serum CRP concentration is specifically explained severe infectious diseases. AutoAbs against IL-6


