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Interleukin (IL)–12 and IL-23 Are Key Cytokines for Immunity against Salmonella in Humans

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Patients with inherited deficiency of the interleukin (IL)–12/IL-23–interferon (IFN)–γ axis show increased susceptibility to invasive disease caused by the intramacrophage pathogens salmonellae and mycobacteria. We analyzed data on 154 patients with such deficiency. Significantly more patients with IL-12/IL-23–component deficiency had a history of salmonella disease than did those with IFN-γ–component deficiency. Salmonella disease was typically severe, extraintestinal, and caused by nontyphoidal serovars. These findings strongly suggest that IL-12/IL-23 is a key cytokine for immunity against salmonella in humans and that IL-12/IL-23 mediates this protective effect partly through IFN-γ–independent pathways. Investigation of the IL-12/IL-23–IFN-γ axis should be considered in patients with invasive salmonella disease.

Immunity against salmonella is complex, but insights may be gained from studies of humans with immunodeficiencies and from animal models of salmonella infection [1]. Such studies suggest the importance of a variety of immunological mechanisms including multiple cytokines [2], particularly the Th1 cytokines [3]; salmonellae, together with mycobacteria (another class of intramacrophage pathogens), commonly cause invasive disease among patients with primary immunodeficiencies characterized by deficiency in the interleukin (IL)–12/IL-23–interferon (IFN)–γ axis [4].

At its simplest, the IL-12/IL-23–IFN-γ axis is believed to consist of 2 complementary components (figure 1): first, an IL-12/IL-23 component, in which, in response to microbial stimuli, macrophages and dendritic cells produce IL-12 and IL-23, which act on NK and T cells and NKT cells [5]; and second, an IFN-γ component, in which IL-12 and IL-23 cause NK, T, and NKT cells to produce IFN-γ, which, in turn, acts on macrophages and other nucleated cells. This results in cellular activation through STAT1 and aids in the elimination of intramacrophage pathogens. Deficiencies have been identified in 5 proteins in this axis: the β1 subunit of the IL-12 and IL-23 receptor (IL-12R/IL-23R), the p40 subunit of IL-12 and IL-23 (IL-12/IL-23), chains 1 and 2 of the IFN-γ receptor (IFN-γR), and STAT1. These deficiencies are caused by mutations in autosomal genes and produce nonfunctional or partially functional proteins [4]. The biology of IL-12 and that of IL-23 are closely entwined with both cytokines sharing the same p40 subunit and their receptors sharing the same β1 subunit [6, 7]. Since the 2 presently described deficiencies in the IL-12/IL-23 component result in a deficiency of both IL-12 and IL-23 signaling, these 2 cytokines must be considered together when studying affected individuals and will, therefore, be referred to as "IL-12/IL-23."

Patients and methods. To compare immunity against salmonellae with immunity against mycobacteria in humans, we reviewed data on 135 patients from 34 countries with confirmed IL-12/IL-23–IFN-γ–axis deficiency whom we have previously investigated and an additional 19 patients described in the medical literature [4, 8]. This encompassed all patients worldwide known to us to have IL-12/IL-23–IFN-γ–axis deficiency at the time of writing, and this process was therefore unselective. Patients had been referred to clinical immunology/infectious disease services, usually with a history of invasive atypical mycobacterial disease with or without salmonella disease or with a family history of

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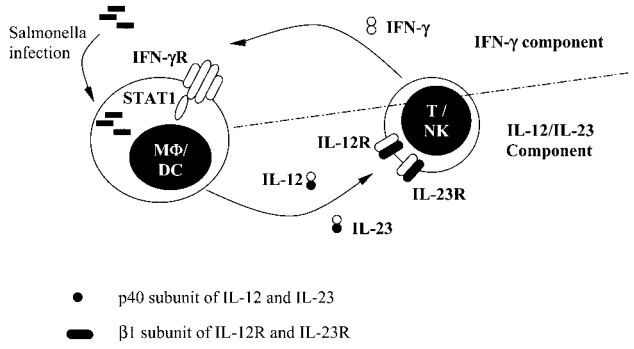

Figure 1. Interleukin (IL)–12/IL-23–interferon (IFN)–γ axis showing (1) the division between IL-12/IL-23 component and IFN-γ component and (2) the proteins that may be absent or deficient in humans: IL-12/IL-23 and IL-12 receptor (R)/IL-23R (in IL-12/IL-23–component deficiency) and IFN-γR and STAT1 (in IFN-γ–component deficiency). For a more comprehensive figure, see [6]. Mϕ/DC, macrophage/dendritic cell; T/NK, T cell/NK cell/NKT cell.

such disease. Only a small minority (6%; 9/154) of patients had been referred with a history of salmonella disease alone. After exclusion of other known immunodeficiencies—including severe combined immunodeficiency, chronic granulomatous disease, AIDS, Di George syndrome, and antibody (IgG, IgA, and IgM) deficiencies—patients were investigated for deficiency in both components of the IL-12/IL-23–IFN-γ axis. No patients were receiving immunosuppressive therapy. We stratified patients according to IL-12/IL-23–IFN-γ–axis deficiency and determined which patients had had salmonella and mycobacterial disease (table 1). In the absence of a suitable control group, the number of patients with IL-12/IL-23–component deficiency and a history of each disease was compared with the number of patients with IFN-γ–component deficiency and the same disease. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated with Epi Info (version 6.04) by use of the exact method.

Results. The median age of patients (or age at death) was similar in both the group with IL-12/IL-23–component deficiency and the group with IFN-γ–component deficiency (9 years [range, 2–35 years] and 11 years [range, 1–62 years], respectively). Surprisingly, we observed that 31 (44%) of 71 patients with IL-12/IL-23–component deficiency had experienced invasive salmonella disease, compared with only 6 (7%) of 83 patients with IFN-γ–component deficiency. This difference is highly significant (OR, 0.10; 95% CI, 0.03–0.28; $P = .0000004$). In contrast, more patients with IFN-γ–component deficiency (94%; 78/83) had had mycobacterial disease, compared with patients with IL-12/IL-23–component deficiency (77%; 55/71) (OR, 4.55; 95% CI, 1.47–16.67; $P = .0061$). All 9 patients with a history of salmonella disease alone had IL-12/IL-23–component deficiency. These observations are striking and suggest that there are key differences in the immune mechanisms operating against salmonellae and mycobacteria. IL-12/IL-23 appears to be important for immunity against salmonella in humans and appears to operate through IFN-γ–independent, as well as IFN-γ–dependent, mechanisms.

Culture-positive salmonella disease occurred in 37 (24%) of 154 patients. Salmonella was typically isolated from extraintestinal sites, rather than from stool (for all except 1 patient), and disease was often severe, with the referring clinician diagnosing sepsis in almost half of affected patients. Salmonella disease was typically difficult to treat; patients often responded poorly to conventional antibiotic therapy. Recurrent disease was common, suggesting either inadequate courses of treatment or a problem with secondary immunity against salmonella. All except 1 of the serovars isolated were nontyphoidal. The median age at the onset of the first salmonella infection among all 37 patients was 3 years (3 years [range, 1–12 years] for patients with IL-12/IL-23–component deficiency and 1.5 years [range, 1–6 years] for patients with IFN-γ–component deficiency). There was no significant difference in the sex ratio of those patients with IL-12/IL-23–IFN-γ–axis deficiency with a history of salmonella disease ($P > .5$).

Discussion. The high prevalence of salmonella disease among patients with IL-12/IL-23–component, as opposed to IFN-γ–component, deficiency suggests that the fundamental deficiency in these patients is not simply a deficit in IFN-γ production. Although many of the actions of IL-12/IL-23 are mediated through IFN-γ, it has other biological functions, and the present study should prompt further investigation into which of these are important for immunity against salmonella in humans. Of the many cytokines implicated in immunity against salmonella, tumor necrosis factor (TNF)–α and granulocyte-macrophage colony-stimulating factor (GM-CSF) are particularly good candidates for mediating IFN-γ–independent ac-

Table 1. Statistical analysis of salmonella and mycobacterial disease in 154 patients with interleukin (IL)–12/IL-23–interferon (IFN)–γ–axis deficiency, stratified according to component deficiency.

				History of salmonella disease				History of mycobacterial disease			
Deficiency	No. of patients	Age, median (range), years	Sex, M:F	Yes, no. (%)	No, no. (%)	OR (95% CI)[a]	P[b]	Yes, no. (%)	No, no. (%)	OR (95% CI)[a]	P[b]
IL-12/IL-23 component	71	9 (2–35)	29:42	31 (44%)	40 (56%)	1		55 (77%)	16 (23%)	1	
IFN-γ component	83	11 (1–62)	39:44	6 (7%)	77 (93%)	0.10 (0.03–0.28)	.0000004	78 (94%)	5 (6%)	4.55 (1.44–16.33)	.0061

[a] Exact method.
[b] Yate's corrected χ^2.

tions of IL-12/IL-23, since both cytokines are produced by T cells and/or NK cells in response to IL-12/IL-23 and both activate macrophages [6, 7]. Animal models suggest a role for both TNF-α [9] and GM-CSF [10] in immunity against salmonella. At present, there is no recognized human immunodeficiency of these cytokines or their receptors, although humans treated with the anti–TNF-α antibody, infliximab, do not appear to show increased susceptibility to salmonella [11]. GM-CSF has been used as an adjunctive therapy against infection in clinical trials, but a beneficial effect is, so far, unproven [12]. Another potential IFN-γ–independent action of IL-12/IL-23 is its immunoregulatory role of promoting Th1 responses, which are critical for effective cell-mediated immunity [6, 7].

Much of our current knowledge of the biology of IL-12/IL-23 comes from animal studies, which also show a role for IL-12/IL-23 in immunity against a wide variety of infections, including those caused by viruses, bacteria, protozoa, and fungi [6]. In contrast, salmonellae and mycobacteria appear to be the only pathogens to which humans with IL-12/IL-23–component deficiency show increased susceptibility, emphasizing the importance of studying immunity against these diseases in humans [13]. Although the present study points to the importance of IL-12/IL-23, this cytokine does not appear to be essential for immunity against salmonella in all patients: we found high titers of antisalmonella antibodies as evidence of exposure to salmonella in 4 of 18 patients with IL-12/IL-23–component deficiency who did not have a history of salmonella disease [8]. This difference in susceptibility to salmonella among patients with IL-12/IL-23–component deficiency requires further investigation. Other patients who did not have a history of salmonella disease may not have been exposed to salmonella. We also found high titers of antisalmonella antibodies in 10 of 12 patients with IL-12/IL-23–component deficiency and a history of salmonella disease [8], indicating that these patients can mount satisfactory antibody responses to salmonella.

In conclusion, clinicians should consider the possibility of an underlying IL-12/IL-23–IFN-γ–axis deficiency in patients with recurrent extraintestinal salmonella disease, as well as in those with disseminated atypical mycobacterial disease. Such deficiencies are probably underdiagnosed in patients with salmonella disease but are clinically significant, as infections often require extended treatment, and live bacterial vaccines must be avoided. The clinical observation that severe salmonella disease is more likely in patients with IL-12/IL-23–component deficiency than in patients with IFN-γ–component deficiency suggests that IL-12/IL-23 is a key cytokine for immunity against salmonella in humans and merits both further investigation into possible IFN-γ–independent IL-12/IL-23–driven mechanisms of immunity and dissection of the contributory role of IL-12 and IL-23. It also suggests a possible role for recombinant IL-12/IL-23 as immunotherapy for severe salmonella disease.

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