Immunity against Helicobacter pylori: Significance of Interleukin-4 Receptor α Chain Status and Gender of Infected Mice

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Vaccination of interleukin-4 (IL-4) receptor α (IL-4Rα) chain-deficient BALB/c mice with Helicobacter pylori urease and cholera toxin or with urease-expressing, live attenuated Salmonella enterica serovar Typhimurium cells revealed that protection against H. pylori infection is independent of IL-4- or IL-13-mediated signals. A comparison of male and female mice suggests a sexual dimorphism in the extent of bacterial colonization that is particularly evident in the absence of the IL-4Rα chain.

Induction of immunity and protection against H. pylori afforded by vaccination with either CT in combination with H. pylori urease or with recombinant urease-expressing, araA attenuated live Salmonella enterica serovar Typhimurium SL3261/pYZ97 cells (6).

For this purpose, IL-4Rα chain-deficient BALB/c ES cell-derived mice (IL-4Rα<sup>-/-</sup>) (11), which are not able to respond to IL-4 or IL-13, and normal female BALB/c mice were vaccinated at weekly intervals with four intragastric applications of 10 µg of CT (Sigma) mixed with 5 µg of native urease purified from H. pylori strain 26695 as previously described (4). The animals were challenged intragastrically 1 week later with 10<sup>9</sup> CFU of mouse-adapted, streptomycin-resistant H. pylori P76 (a kind gift from H. Kleanthous; OraVax) grown to late log phase at 37°C with shaking at 200 rpm in an atmosphere of 5% O<sub>2</sub>, 85% N<sub>2</sub>, and 10% CO<sub>2</sub> in brain heart infusion broth (Becton

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FIG. 1. Vaccination with purified urease in combination with CT is less immunogenic but still protective against H. pylori infection in IL-4Rα<sup>-/-</sup> BALB/c mice. Groups of eight BALB/c and IL-4Rα<sup>-/-</sup> mice were immunized with purified native urease in combination with CT and challenged with H. pylori. (A) Urease-specific Ig titer in serially diluted serum were determined by enzyme-linked immunosorbent assay (6) in immunized and challenged BALB/c (●) and IL-4Rα<sup>-/-</sup> (■) mice. Serum of nonimmunized, infected controls (△) was used for comparison. (B) Urease activity associated with gastric tissue was determined by colorimetry after 24 h of incubation in 330 mM urea and 1 mM phosphate buffer (pH 6.9)-phenol red (0.001% [wt/vol]). Values represent means ± standard errors of the means (n = 8). OD<sub>520nm</sub>, optical density at 420 nm.
However, in our hands IL-4Rα2 or -negative linked immunosorbent assay using plate-bound urease-positive immunoglobulin (Ig) in serum were determined by enzyme-immunogenicity of the vaccine, relative titers of urease-specific previously (6). In order to assess a general parameter of the immune response, IL-4Rα2 luted serum of BALB/c (F) mice were challenged with H. pylori (Fig. 2A). (A) Urease-specific Ig levels in serially diluted serum of BALB/c (●), IL-4Rα2/− (■), and control (△) mice (A) and urease activity (B) were measured as described for Fig. 1. OD420nm optical density at 420 nm. (C) The numbers of H. pylori cells colonizing gastric tissue were estimated by quantitative culture of serial dilutions of bacteria suspended by vortexing from weighed tissue fragments in brain heart infusion medium. The values shown are geometric means of CFUs determined from five mice per group ± standard errors of the means.

FIG. 2. Vaccination with urease-expressing, recombinant Salmonella SL3261/pYZ97 is equally immunogenic in IL-4Rα−/− and BALB/c mice and protects against H. pylori infection. Mice were immunized with the indicated numbers of CFU of SL3261/pYZ97 and challenged with H. pylori. (A) Urease-specific Ig levels in serially diluted serum of BALB/c (●), IL-4Rα2/− (■), and control (△) mice (A) and urease activity (B) were measured as described for Fig. 1. OD420nm optical density at 420 nm. (C) The numbers of H. pylori cells colonizing gastric tissue were estimated by quantitative culture of serial dilutions of bacteria suspended by vortexing from weighed tissue fragments in brain heart infusion medium. The values shown are geometric means of CFUs determined from five mice per group ± standard errors of the means and are representative of three experiments with similar results.

Dickinson) containing 10% fetal calf serum (Gibco), supplemented with 400 μg of streptomycin/ml. Three weeks later, mice were bled and killed, and their stomachs were removed. Stomachs were cut in half to determine in parallel tissue-associated urease activity and H. pylori burdens as described previously (6). In order to assess a general parameter of the immunogenicity of the vaccine, relative titers of urease-specific immunoglobulin (Ig) in serum were determined by enzyme-linked immunosorbent assay using plate-bound urease-positive or -negative H. pylori lysates (6). Immunized and challenged IL-4Rα−/− mice had fivefold-lower Ig titers specific for urease than BALB/c mice (P < 0.003 in a t test), confirming that IL-4 and IL-13 are important factors in the induction of this immune response by CT and urease (Fig. 1A). This was also reflected in lower proliferation by spleen cells from vaccinated IL-4Rα−/− mice than by those from wild-type animals in the presence of urease-containing H. pylori lysates (not shown). However, in our hands IL-4Rα− mice could still be protected from challenge infection with this treatment since no

Next, IL-4Rα−/− and BALB/c female mice were vaccinated orally with 107 or 109 CFU of SL3261/pYZ97 as described previously (6). Five weeks later vaccinated and control animals were challenged intragastrically with 109 CFU of H. pylori P76. In contrast to immunization with CT, vaccination with SL3261/pYZ97 induced similar levels of urease-specific Ig in wild-type and IL-4Rα−/− mice (Fig. 2A). While the total IgG levels were equivalent, no urease-specific IgG1 was detectable in IL-4Rα−/− mice in contrast to what was found for wild-type BALB/c mice (data not shown). Immunization of BALB/c mice with 107 CFU instead of 109 CFU of SL3261/pYZ97 is not protective in every individual. A difference in vaccine efficacy at these doses between BALB/c and IL-4Rα−/− mice, in particular at the lower dose, should reveal a role if any for IL-4 and IL-13 signaling in protection. However, the reductions of bacterial burdens in BALB/c and IL-4Rα−/− animals were similar (Fig. 2B and C), confirming the results shown in Fig. 1B.

Control infected IL-4Rα−/− female mice tended to have lower levels of H. pylori colonization than infected female BALB/c mice (Fig. 1 to 3); however, this difference was not statistically significant (P > 0.1). This result is consistent with the recent reporting of similar burdens in IL-4 deficient and wild-type C57BL/6 mice (1) but is at odds with data of an earlier study showing increased bacterial colonization in IL-4-deficient mice (10). It is of note that female mice were infected here and in the former study while male animals were used in the latter. Interestingly, based on urease activity and CFU determinations male mice on average suffered from a higher bacterial burden in IL-4Rα−/− mice than BALB/c mice (P < 0.018 by t test [probability that this difference is due to chance]) and may account for the observed discrepancies between the aforementioned studies. A difference between genders with respect to the extent of H. pylori colonization was also found when comparing the efficiencies of SL3261/pYZ97 vaccination in male and female BALB/c mice (Fig. 4). Vaccination with 107 or 109 CFU of SL3261/pYZ97 against H. pylori challenge was less
efficacy in males (Fig. 4B). This is unlikely to reflect lower immunogenicity of the vaccine in males since levels of urease-specific antibodies were similar in both sexes (Fig. 4A).

In summary, our results show that IL-4 and IL-13 are not critical for the CD4 T-cell-mediated mechanism responsible for H. pylori clearance in vaccinated animals. Differences noted between normal and IL-4/IL-13 deficiency mice in vaccination experiments likely reflect the dependence of the immune reaction on IL-4 during the induction phase when CT is used (12). Furthermore a sexual dimorphism in the degree of colonization by H. pylori, which may represent a novel factor contributing eventually to the higher prevalence of gastric cancer seen in male patients, is described (3).

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REFERENCES


