High Constitutive Interleukin 10 Level Interferes With the Immune Response to Varicella-Zoster Virus in Elderly Recipients of Live Attenuated Zoster Vaccine

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Introduction. Live attenuated zoster vaccine (Zostavax) was used to test the hypothesis that constitutive level of interleukin 10 (IL-10), which may be high in elderly subjects, impairs vaccine efficacy. If constitutive IL-10 impairs vaccine efficacy, the effectiveness of viral vaccines might be improved by transient inhibition of IL-10 before vaccination.

Methods. Zostavax was given to 26 patients (age, 60–80 years). IL-10 and immunity to varicella zoster virus (VZV) were measured at baseline and after vaccination. Fluorescent antibody to membrane antigen (FAMA) assays and glycoprotein enzyme-linked immunosorbent assays (gpELISAs) were used to assess humoral immunity; anti-varicella virus T-cell responses were studied in a subset of subjects. In a prospective animal model, T-cell responses to chimeric vaccines against lymphocytic choriomeningitis virus (LCMV) were assessed in mice that express or lack IL-10.

Results. FAMA assays revealed significant boosting (by 4-fold) of humoral immunity, which occurred only in subjects (10 of 26) with a low constitutive IL-10 level (ie, <20 pg/mL); moreover, the Zostavax-induced FAMA and gpELISA responses were inversely related to the constitutive IL-10 level. Significant VZV-specific T-cell responses followed vaccination only in subjects with a low constitutive IL-10 level. Vaccine-induced LCMV-specific T-cell responses in mice lacking IL-10 were greater than in wild-type animals.

Conclusions. A high constitutive IL-10 level adversely affects vaccine efficacy.

Keywords. Varicella; herpes zoster; vaccination; vaccine failure; cytokine; humoral immunity; FAMA; IL-10; T cells.

Live attenuated zoster vaccine (Zostavax) is licensed for protection against herpes zoster (HZ) and postherpetic neuralgia. This vaccine is similar to but more potent than the vaccine that prevents primary varicella zoster virus (VZV) infection [1]. A dose of VZV that is 1/14th that in Zostavax is highly effective in protecting susceptible children from varicella (85% after 1 dose and 98% after 2 doses) [2, 3]; however, Zostavax protects only 50%–65% of healthy adults (age, 60–80 years) from HZ [4]. Evidence suggests that immune responses of younger subjects to Zostavax are better than those of elderly adults [5]; nevertheless, it is still unclear why Zostavax protects relatively few recipients from HZ while varicella vaccine achieves nearly universal protection against varicella.

Inadequate immune responses to hepatitis and influenza vaccines have been attributed to high preexistent (constitutive) levels

of IL-10 [6, 7]. We therefore tested the hypothesis that a high constitutive IL-10 level in elderly subjects impedes the development of a vigorous immune response to VZV. Antibody levels and T-cell responses to VZV were measured after Zostavax vaccination, to evaluate the vigor of the immune response. Antiviral T-cell responses are thought to be responsible for protection against HZ [8, 9], which is due to reactivation of latent VZV. In contrast, humoral immunity protects against the primary VZV infection that causes varicella [10]. Because vaccination stimulates both humoral and cellular immunity, the postvaccination increase in antibody titer has been found to provide a valuable estimate of the vigor of the total immune response [11]. We therefore evaluated the vaccine-induced change in levels of antibody to VZV to test the prediction that the strength of this phenomenon would vary inversely with the constitutive IL-10 level. Release of interferon γ (IFN- γ) from T cells was also assayed in a subset of subjects. Two methods were used to evaluate humoral immunity. One was the fluorescent antibody to membrane antigen (FAMA) assay [12], which yields results that correlate with protection from varicella and is accepted as the gold standard against which other methods to measure protective immunity are compared [13]. The other was the glycoprotein enzyme-linked immunosorbent assay (gpELISA), which yields results

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that do not correlate with protection but is the method most commonly used to assess immunity to VZV [10, 11].

METHODS

We measured IL-10 levels before and 1 day after injection of Zostavax in 26 individuals aged 60–80 years (mean age, 67 years; median age, 67 years [interquartile range, 64–69 years]; sex, female for 18 and male for 8) who were immunized at Columbia University Vagelos College of Physicians and Surgeons. All patients were in good health, and none were immunocompromised. The most common medications were statins (14 subjects) and antihypertensive agents (10 subjects); 8 subjects took no drugs regularly. One subject had type 2 diabetes controlled with metformin. A blood specimen (volume, 20 mL) was collected on the day of vaccination and 1 day and 6 weeks later. Laboratory testing occurred at Columbia University (New York, NY) and at the Division of Allergy and Immunology at Scripps Green Hospital (La Jolla, CA).

FAMA assays and gpELISAs were used to evaluate humoral immunity to VZV before and 6 weeks after receipt of Zostavax. The FAMA assay uses doubling dilutions of serum to provide an antibody titer [14], while the gpELISA (performed at Merck) [5, 11] provides continuous data, rather than discontinuous titers. Levels of IL-10 were measured by standard ELISAs, using plates from R&D Systems (Minneapolis, MN) [15, 16]. Cells were collected at Columbia University and shipped to Scripps Green Hospital for analysis. T-cell activation was studied in 10 of 26 individuals who, on screening prior to vaccination, displayed low (5) or high (5) constitutive levels of IL-10. Samples from 16 individuals did not survive shipment. Samples (3) with poor cell survival were discarded. Release of IFN- γ from T cells was measured as described previously [17, 18] by using an enzyme-linked immunospot (ELISPOT) assay and by zoster peptide stimulation in combination with a fluorescence-activated cell sorting (FACS) assay. Cells from 4 individuals with low and 3 with high constitutive IL-10 levels were successfully analyzed. Similar results were obtained from each of the 4 samples from subjects with low constitutive IL-10 levels. Three of these were randomly selected for comparison to the 3 samples from subjects with high constitutive levels of IL-10.

The importance of IL-10 in vaccine efficacy was examined further in a mouse model in which IL-10 is expressed or deleted and chimeric viruses are used for vaccination [15, 19]. Chimeric viruses were vesicular stomatitis virus or influenza virus, each expressing the lymphocytic choriomeningitis virus (LCMV) glycoprotein gene. Mice were maintained in pathogen-free conditions. Handling conformed to the requirements of the National Institutes of Health, the Scripps Research Institute's Institutional Animal Care and Use Committee, and AAALAC International. Mice lacking IL-10 were purchased from Jackson Laboratory (Bar Harbor, ME).

RESULTS

Constitutive IL-10 levels on the day of vaccination were variable and positively skewed; the overall mean level ($\pm$ SD) was 384 ± 205 pg/mL, but the median value was 54 pg/mL, and the coefficient of variation was 272%. The IL-10 level on the day after vaccination was significantly increased (mean [$\pm$ SD], 794 ± 272 pg/mL; $P = .01$, by the Wilcoxon matched pairs signed rank test); however, the level of IL-10 for each individual on the day after vaccination was highly correlated with that measured in the blood specimen collected on the previous day (coefficient of correlation, 0.98; $P < .0001$). IL-10 levels in the current set of subjects were not significantly correlated with age (Spearman $r = -0.0134$; $P = .9482$). Fourteen individuals had IL-10 levels of ≤ 20 pg/mL at the time of vaccination, with a mean value ($\pm$ SD) of 2 ± 1 pg/mL; this group, as in other studies of controls [20, 21], was defined as having a low constitutive IL-10 level. Twelve of 26 individuals had IL-10 levels of >20 pg at the time of vaccination (mean [$\pm$ SD], 821 ± 418 pg/mL); this group was defined as having a high constitutive IL-10 level. No relationship was found between the use of statins or antihypertensive agents and constitutive levels of IL-10.

Vaccination was found to enhance immunity significantly in the overall population of vaccinees, no matter whether the FAMA assay or gpELISA was used for evaluation. For the FAMA assay, the geometric mean ratio ($\pm$ SD) of the postvaccination titer to the prevaccination titer was 2.29 ± 0.07 ($P < .001$, by the paired t test). The mean number of gpELISA units ($\pm$ SD) rose from 419 ± 111 to 705 ± 176 ($P < .01$, by the paired t test). A boost in the FAMA titer was defined as a ≥ 4 -fold increase 6 weeks after vaccination. A ≥ 3 -fold increase in the number of gpELISA units 6 weeks after vaccination was defined as a boost. A significant boost in humoral immunity was revealed by the FAMA assay and/or gpELISA in 11 of 26 patients (46%). Of these, FAMA revealed the boost in 10, and, in 3, the boost was also reflected in increased gpELISA units. In 1 patient, a boost was observed with gpELISA but not with the FAMA assay. In 10 of 11 patients (91%) for whom the FAMA assay and/or gpELISA demonstrated a postvaccination boost in antibody titer, a low constitutive level of IL-10 was found at vaccination ($P < .01$, by χ^2 analysis; Figure 1A). A boost in the FAMA titer was more consistently related to a low constitutive IL-10 level than was a boost in the number of gpELISA units. In each of the 10 patients for whom the FAMA assay revealed a postvaccination boost in immunity, a low constitutive IL-10 level was present at vaccination, and there were no boosts in the FAMA titer among subjects with a high constitutive IL-10 level ($P < .001$, by χ^2 analysis; Figure 1B). In contrast, gpELISA revealed boosts only in 4 subjects, one of whom also had a high constitutive IL-10 level ($P =$ not significant, by χ^2 analysis; Figure 1C). There was a weak correlation between the preexisting and boosted FAMA titers (Spearman $r = 0.611$), but the

Figure 1. Boosting of humoral immunity to varicella zoster virus (VZV) in response to Zostavax in subjects with high or low blood levels of interleukin 10 (IL-10) at the time of vaccination. Blood levels of IL-10 were determined on the day of vaccination. Two methods, a glycoprotein enzyme-linked immunosorbent assay (gpELISA) and a fluorescent antibody to membrane antigen (FAMA) assay, were used to assess the change in immunity to VZV occurring between the day of vaccination and 6 weeks following vaccination. Boosts in immunity were defined as a ≥ 4 -fold increase in the FAMA titer or gpELISA level. Boosts in immunity are shown as a function of the IL-10 level. An IL-10 level of ≤ 20 pg/mL was considered low, while greater levels were considered high. *A*, Boosting detected by the combination of the FAMA assay and/or gpELISA was significantly related to the existence of low levels of IL-10 at the time of vaccination. *B*, Boosting of FAMA titers were found only in patients with low levels of IL-10. This distribution is highly significant. *C*, Boosting detected only on the basis of gpELISA levels was not significantly related to preexisting levels of IL-10. NS, not significant.

constitutive IL-10 level and the preexisting FAMA titer were not correlated (Spearman $r = -0.094$).

The degree to which vaccination increased humoral immunity to VZV was quantified to determine whether these increases were, as the boosting of immunity suggested, inversely proportional to the constitutive IL-10 level. The postvaccination rise in the FAMA titer was highly significant in patients with a low constitutive IL-10 level ($P < .001$, by the ratio paired t test) but not significant in those with a high constitutive IL-10

level (Figure 2A). When compared directly, the mean increase in FAMA titer in the group with a low constitutive IL-10 level was significantly greater than that in the group with a high constitutive IL-10 level ($P < .001$, by the unpaired t test; Figure 2B). Similar data were obtained with gpELISA, despite the failure of gpELISA to reach the threshold required for boosts in the immunity of individual subjects. Significant postvaccination increases in the number of gpELISA units were found, in both the group with a low constitutive IL-10 level ($P < .001$, by

Figure 2. Zostavax-induced increases in humoral immunity in subjects with low interleukin 10 (IL-10) levels are greater than those in subjects with high IL-10 levels. *A*, Fluorescent antibody to membrane antigen (FAMA) titers at the time of vaccination (before) and 6 weeks after vaccination (after) are shown for subjects with low or high levels of IL-10. Significant increases in the FAMA titer were found only in the group with low IL-10 levels. *B*, The fold increase in FAMA titer that Zostavax induces is significantly greater in patients with low IL-10 levels than in patients with high IL-10 levels. *C*, Glycoprotein enzyme-linked immunosorbent assay (gpELISA) levels at the time of vaccination (before) and 6 weeks after vaccination (after) are shown for subjects with low or high levels of IL-10. Significant increases in gpELISA levels were found in both groups. *D*, The fold increase in gpELISA levels that Zostavax induces is significantly greater in patients with low IL-10 levels than in patients with high IL-10 levels. NS, not significant.

the ratio paired *t* test) and the group with a high constitutive IL-10 level ($P < .02$, by the ratio paired *t* test; [Figure 2C](#)); however, when compared directly, the increase in the number of gpELISA units in the group with a low constitutive IL-10 level was significantly greater than that in the group with a high constitutive IL-10 level ($P < .001$, by the unpaired *t* test; [Figure 2D](#)). These observations indicate that the constitutive IL-10 level at vaccination is a critical, albeit negative, determinant of the vigor

of the humoral immune response to Zostavax. Higher levels of antibodies are induced by Zostavax when it is given to subjects with a low constitutive IL-10 level. The FAMA test provides a better measure of the boosting of immune responses in individuals, although the inhibitory effect of IL-10 is revealed both by the FAMA assay and gpELISA in populations of vaccinees.

The zoster peptide stimulation FACS assay [17, 18] used VZV antigens obtained from Donald Gilden. A memory

Figure 3. The constitutive interleukin 10 (IL-10) level is inversely related to the generation of varicella zoster virus (VZV)-specific antibodies, as well as to CD8 $^{+}$ and CD4 $^{+}$ T-cell counts. T-cell counts were determined by peptide stimulation with VZV antigens, and intracytoplasmic concentrations of interferon- γ (IFN- γ) were measured by fluorescence-activated cell-sorting analysis. *A*, Cell numbers are plotted against intensities of IFN- γ fluorescence (vertical axis), with control CD8 $^{+}$ T cells in the upper panels and control CD4 $^{+}$ T cells in the lower panels. Phorbol myristate acetate (PMA) and VZV antigens applied to cells from a positive-control subject induced CD8 $^{+}$ and CD4 $^{+}$ T cells to express IFN- γ . No IFN- γ response was seen when VZV antigens were omitted (negative). *B*, Cells isolated from a subject with a constitutive IL-10 level of 252 pg/mL at vaccination. The fluorescent antibody to membrane antigen (FAMA) titer was 1:8 at vaccination and 1:8 six weeks later. VZV antigens failed to induce IFN- γ expression in either CD8 $^{+}$ or CD4 $^{+}$ T cells. *C*, Cells isolated from 2 subjects with a constitutive IL-10 level of 0 pg/mL at vaccination. The FAMA titer was 1:16 (*C*₁) and 1:4 (*C*₂) at vaccination but rose to 1:256 (*C*₁) and 1:32 (*C*₂) 6 weeks later. In both subjects, after 6 weeks, VZV antigens induced IFN- γ expression in both CD8 $^{+}$ and CD4 $^{+}$ T cells. *D*, Enzyme-linked immunospot (ELISPOT) assays revealed the numbers of virus-specific CD8 $^{+}$ T cells that make IFN- γ , in correlation with both IL-10 and FAMA levels. Three positive responders (*D*₁) are shown in the columns at the left, and 3 negative responders (*D*₂) are shown in the columns on the right. For each individual patient, the constitutive IL-10 levels and the initial FAMA titers at the time of vaccination and after 6 weeks are indicated. The numbers of IFN- γ -expressing cells are also shown for each illustrated ELISPOT assay (lower right of each Petri dish). Note that the constitutive IL-10 level was low and the FAMA titer increased at least 4-fold in each positive responder, while the constitutive IL-10 level was high and the FAMA titer failed to increase in each negative responder.

IFN- γ response to stimulation with phorbol myristol acetate and VZV peptides was seen in control CD8⁺ and CD4⁺ T cells (Figure 3A). When VZV peptides were used for stimulation and FACS analysis was used to investigate CD8⁺ and CD4⁺ T cells obtained after administration of Zostavax, a significant level of IFN- γ expression occurred in 4 of 4 subjects with low constitutive IL-10 levels and a ≥ 4 -fold vaccination-induced increase in FAMA. The expression of IFN- γ in neither CD8⁺ nor CD4⁺ T cells increased in 0 of 3 subjects when the constitutive IL-10 level was high (Figure 3B), but expression of IFN- γ in both CD8⁺ and CD4⁺ T cells was always increased (in 4 in 4 subjects) significantly when the constitutive IL-10 level was low (Figure 3C1 and 3 C2). The consistency of the relationship of T-cell activation to the preexisting level of IL-10 was significant ($P < .03$, by the Fisher exact test). T-cell production of IFN- γ prior to vaccination was also compared to that determined by ELISPOT assays performed 6 weeks after administration of Zostavax (Figure 3D). In these assays, 3 of 3 individuals displaying a low constitutive IL-10 level at vaccination had a ≥ 3 -fold increase in the number of T cells that released IFN- γ (Figure 3D1). In contrast, 3 of 3 individuals displaying a high constitutive IL-10 level at vaccination did not have an increased number of T cells making IFN- γ (Figure 3D2).

Humans with genetic depletion of IL-10 are not available; therefore, the inverse correlation of the constitutive IL-10 level with the postvaccination immune response is necessarily associative. To enhance the interpretation of our results, we used an *Il10*^{-/-} mouse model [15]. To test the hypothesis that deficient constitutive IL-10 signaling enhances postimmunization immune responses, *Il10*^{+/+} and *Il10*^{-/-} mice were vaccinated with a chimeric vesicular stomatitis virus expressing the LCMV glycoprotein gene (given intravenously) or a chimeric influenza virus expressing the LCMV glycoprotein gene (given intramuscularly). *Il10*^{-/-} mice, vaccinated with the chimeric viruses, were compared to similarly vaccinated *Il10*^{+/+} littermates. Significantly greater virus-specific CD8⁺ and CD4⁺ T-cell responses occurred in *Il10*^{-/-} mice than in *Il10*^{+/+} mice (Figure 4).

DISCUSSION

The current study was undertaken to investigate the role that the variable constitutive levels of IL-10 we found in a population of elderly humans plays in determining the vigor of immune responses to the live attenuated HZ vaccine (Zostavax). Although this vaccine was revolutionary in that it provided the first effective means of preventing HZ, it is disappointing in protecting a limited proportion of elderly individuals (50%–65%) at highest risk to develop HZ [4]. This limitation has led to the introduction of a subunit vaccine (Shingrix) that protects a far greater proportion of recipients (91.3%) but that uses the AS01_B adjuvant system that enhances humoral and CD4⁺ T-cell responses to subunit antigens from a number of pathogens [22].

Although studies, including a large meta-analysis, have not revealed serious adverse events associated with AS01_B or similar adjuvants [23], caution involving their use has been advised because adjuvanted vaccines might, in theory, increase the frequency of autoimmune disorders [24]. The subunit vaccine, moreover, requires 2 doses and, probably because of the adjuvant, is relatively reactogenic [22–24]. Zostavax, therefore, is still in use and might become more valuable if its efficacy could be improved. The potential for IL-10 to limit the efficacy of vaccines, moreover, has a significance that transcends its specific interaction with Zostavax and may extend to other vaccines, including Shingrix.

Observations were all consistent with the hypothesis that elevated IL-10 levels at the time of vaccination negatively affect responses to Zostavax in elderly recipients. Several means of evaluating the vigor of the humoral immune response were used. Boosts in immunity were measured and antibody levels quantified using FAMA assays and gpELISAs. A significant vaccine-induced boost in immunity (a >4 -fold increase in the FAMA titer and a >3 -fold increase in the number of gpELISA units) was revealed only by FAMA and occurred only in patients with low constitutive levels of IL-10. An increase in antibody titer was detected both with the FAMA assay and the gpELISA, and, with both, the vaccine-induced increment in immunity was significantly greater in individuals with a low constitutive level of IL-10 than in individuals with a high constitutive IL-10 level. Observations of cellular immunity, albeit in a small number of patients, were consistent with those obtained with FAMA. Again, ELISPOT assays revealed a Zostavax-induced increase in IFN- γ levels only in T cells from patients with low constitutive levels of IL-10; moreover, in each of these individuals Zostavax also caused at least a 4-fold increase in the FAMA titer. Similarly, zoster peptide stimulation FACS assays also revealed that CD8⁺ and CD4⁺ T cells became responsive after Zostavax only in individuals in whom low levels of constitutive IL-10 were present and in whom a boost also occurred in the FAMA titer. A further prospective test of the hypothesis that deficient IL-10 signaling at the time of administration of antigen promotes a vigorous immune response to vaccination was done in mice lacking IL-10. These mice and wild-type controls were vaccinated with the LCMV glycoprotein expressed in chimeric vesicular stomatitis or influenza viruses. Substantially greater virus-specific CD8⁺ and CD4⁺ T-cell responses occurred in mice in which genes encoding IL-10 were deleted than in wild-type controls. These data, from multiple tissue specimens in the tractable murine model, again suggest that IL-10 signaling at the time of immunization is counterproductive to the induction of a vigorous cellular immune response.

Our observations with zoster vaccination are similar to the weak immune responses to influenza [6] and hepatitis B [7] vaccines in elderly individuals that also directly correlate with high levels of IL-10. IL-10 has been implicated in poor immune

Figure 4. Wild-type (wt) and *Il10*^{-/-} mice were used to quantify the effect of interleukin 10 (IL-10) on virus-specific T-cell responses after vaccination. *A*, *Il10*^{-/-} and wt mice were vaccinated with vesicular stomatitis virus expressing lymphocytic choriomeningitis virus (LCMV) glycoprotein (GP), and after a week the numbers of splenic LCMV-specific CD8⁺ T cells and CD4⁺ T cells expressing interferon γ (IFN- γ ; vertical axis) were determined. Significantly more LCMV-specific CD8⁺ and CD4⁺ T cells were activated in *Il10*^{-/-} than in wt mice. *B*, Mice were vaccinated with influenza virus expressing LCMV GP, and after a week the numbers of LCMV-specific CD8⁺ T cells expressing IFN- γ (vertical axis) were determined in the lungs and lymph nodes. Significantly more LCMV-specific CD8⁺ T cells in both the lungs (4-fold) and lymph nodes (22-fold) were activated in *Il10*^{-/-} mice than in wt mice. * $P < .05$.

responses to hepatitis B virus, possibly leading to chronic hepatitis [7, 25]. Similarly, elevated levels of IL-10 during infections with hepatitis C virus has been proposed to contribute to its persistence [26].

IL-10 levels have been reported to increase as a function of age [27, 28], although this was not apparent in the current sample, which was limited to elderly subjects. Studies with Zostavax have demonstrated that the extent of protection it affords is inversely related to the age of individuals at the time of their vaccination [4]. Children vaccinated with live attenuated VZV (vOka strain) have excellent immune responses to VZV even though the varicella vaccine used in children is 14-fold weaker

than that used to vaccinate adults against zoster. A formal study that relates constitutive levels of IL-10 to the efficacy of the live attenuated varicella vaccine in children has not been conducted; however, it is tempting to speculate that, with respect to vaccination, children are on the favorable side of the relationship between age and IL-10 level.

IL-10 is a multifunctional cytokine that limits the magnitude of immune responses to viruses and modulates (ie, shuts off) the immune response as viral infections clear [29]. By regulating the intensity of immune responses, associated tissue injury due to immunopathology or autoimmune disease is limited. IL-10 can be produced by and signal through many cells of the immune

system, including dendritic cells, B cells, macrophages, CD4⁺ T cells, CD8⁺ T cells, and innate and adaptive regulatory T cells [29]. IL-10 aborts T-cell responses when present during priming, and IL-10 can inhibit ongoing T-cell activity. IL-10 also acts directly on antigen-presenting cells to prevent maturation, decrease stimulatory molecule expression (ie, major histocompatibility complex class I and class II, B7-1, and B7-2), and inhibit T-cell activation [29]. Under steady-state conditions, IL-10 prevents circulating, self-reactive T cells from giving rise to autoimmune disease. Consistent with the role of controlling autoimmune reactions, mice lacking IL-10 develop autoimmune colitis [30]. The genetics of IL-10 suggest that its expression may be related to the manifestation of HZ. The promoter region of IL-10 contains 3 single-nucleotide polymorphisms (G/A at -1082, C/T at -819, and C/A at -592); they form 3 common haplotypes, GCC, ACC, and ATA, respectively [31]. The ATA haplotype is overrepresented in patients with HZ [32], although it is probably also associated with decreased IL-10 transcription [31]. It may be, therefore, that the increased expression of proinflammatory cytokines, rather than the inability to mount an immune response, explains the overrepresentation of the ATA haplotype in patients with HZ.

One possible approach to improving the immune response after administration of Zostavax or any other vaccine that is not as efficient as needed would be to block IL-10 function transiently, possibly by passive immunization with antibodies to IL-10. Transient blockade of IL-10 would not be expected to lead to autoimmunity in humans. No evidence of autoimmune responses or autoimmune disease during transient blockade of IL-10 has been observed in animals [15, 16]. Another approach might be to antagonize IL-10 locally, at the site of immunization. For example, the adjuvant AS01B, which is used in Shingrix, boosts both innate and adaptive immunity to VZV [22, 33]. This adjuvant acts locally in lymph nodes. It is not known whether AS01B interacts with IL-10, but it is conceivable that locally restricted anti-IL-10, which does not circulate, might similarly boost the efficacy of immunization.

It has been noted that IL-10 is induced during persistent infection with LCMV, but its removal, either genetically, in *Il10*^{-/-} mice, or through administration of antibodies to the IL-10 receptor, resurrects antiviral CD4⁺ and CD8⁺ T-cell function in LCMV-infected animals [15, 16]. Once restored, functional T cells can control LCMV infection [15, 16]. Importantly for our report, blockade of IL-10 in mice allowed an otherwise ineffective therapeutic DNA vaccine against LCMV to stimulate antiviral immunity sufficiently to clear persisting LCMV. Blockade of IL-10 enhances the efficacy of the LCMV vaccine by increasing specific antiviral T-cell numbers and responses [15]. These findings of the role for IL-10 in suppressing immune responses to LCMV [15, 16] have independently been confirmed [34, 35] and have now been extended to human immunodeficiency virus, influenza virus [6], and hepatitis C virus [36] infections, with influenza virus and vesicular stomatitis virus addressed in

the current study. The mouse experiments using mice lacking IL-10 or its receptor, therefore, provide strong genetic evidence of both the association of IL-10 with the immune response and, more importantly, that transient blockade of IL-10 significantly enhances vaccination against a variety of viruses.

Zoster is thought to be under the control of cellular immunity. It has recently been recognized, however, that VZV antibody titers may not be meaningless regarding analysis of immune responses following immunization against zoster [37]. In one study, a 5-fold rise in VZV antibody titers from the time before to 6 weeks after immunization was an excellent predictor of protection from zoster. Vaccine efficacy was negligible for vaccinees with no antibody rise; however, the efficacy was 90% in vaccinees, with a >5-fold rise in the antibody titer [37]. These data are in good agreement with the current observation that constitutive IL-10 existing at the time of vaccination similarly affects the vigor of the humoral immune response measured by the FAMA assay, which is a correlate of immunity to varicella, and the cellular response to Zostavax. It is thus possible that the FAMA titer provides a correlate of immunity to HZ, as well as to varicella. In addition, antiviral antibodies reduce the titers of viruses, thereby limiting the exhaustion of T cells and promoting their function for clearing virus to terminate acute or persistent infection. It therefore appears that a poor antibody response (at least that measured by the FAMA assay) and poor cell-mediated response to live attenuated zoster vaccine by IL-10 may indicate a poor degree of protection. A high constitutive IL-10 level in a substantial minority of vaccinated patients is likely also responsible for the disappointing protection against HZ with the live attenuated zoster vaccine. The AS01B-adjuvanted Shingrix vaccine is superior to Zostavax and results in >90% protection, even in individuals >70 years of age [33]. In the future, it will be not only be interesting but important to determine the effect of AS01B on IL-10 signaling. It will also be of importance to determine whether IL-10 responses in elderly individuals who receive vaccines predict the efficacy of the vaccine and whether alternative approaches can be found to provide vaccine protection when IL-10 expression appears to be an obstacle.

Notes

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