treated with red light or not) were placed, one at a time, in front of a Dolan-Jenner Fiber Illuminator (Woburn, MA) provided with an Ealing blue filter used to induce phototropic curvature. On one side of the tray (90° from the blue light beam), a TV camera (Javelin Electronics, Torrance, CA) was placed. This camera was connected to a personal computer equipped with a Jandel Video Analysis Software (Corte Madera, CA). Images were saved every 10 min after the blue light pulse (1 μmol m^{-2} s^{-1} for 200 s), in which at least 25 coleoptiles were identified and their curvature at the tip was measured. All experiments were conducted in a dark room, and whenever light was necessary to obtain a clear image of the coleoptiles, a low-intensity green light source, having no overlap with the blue light spectrum, was used.
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The Role of Interleukin-6 in Mucosal IgA Antibody Responses in Vivo

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In mice with targeted disruption of the gene that encodes interleukin-6 (IL-6), greatly reduced numbers of immunoglobulin A (IgA)–producing cells were observed at mucosae and grossly deficient local antibody responses were recorded after mucosal challenge with either ovalbumin or vaccinia virus. The IgA response in the lungs was completely restored after intranasal infection with recombinant vaccinia viruses engineered to express IL-6. These findings demonstrate a critical role for IL-6 in vivo in the development of local IgA antibody responses and illustrate the effectiveness of vector-directed cytokine gene therapy.

Immune responses at mucosal surfaces are characterized by production and secretion of antibodies of the IgA isotype, which represent a first line of defense against colonization by many pathogens (*1*). Most IgA antibody-containing cells (ACCs) originate in mucosa-associated lymphoid tissue (MALT), such as Peyer's patches in the intestine, where they encounter antigen. Subsequently, ACCs disseminate by means of draining lymph and blood circulation to mucosal effector sites where IgA production occurs (*1, 2*). Efforts to design effective mucosal vaccines, given impetus by the current human immunodeficiency virus (HIV) epidemic (*3*), have been hampered by a lack of information regarding mucosal immunoregulation. Although the induction of mucosal IgA responses is known to be dependent on cognate help provided by $CD4^+$ T cells in MALT (*4*), factors important in the subsequent development of these responses have not been well defined. Interleukin-6, a multifunctional cytokine originally identified for its ability to induce B cell terminal differentiation (*5, 6*), markedly and selectively enhances IgA production in vitro by isotype-committed B cells (*7, 8*). The in vivo relevance of these findings has not been determined, but the presence in mucosal tissues of T cells, macrophages, and other cells capable of IL-6 production in vitro (*1, 8, 9*), and the broad distribution of cells containing IL-6 mRNA in intestinal mucosa (*10*), are consistent with the proposition that this factor is important in regulating the effector stage of IgA responses.

To obtain evidence for this hypothesis, we studied mucosal IgA antibody responses in mice rendered deficient in IL-6 production ($IL\text{-}6^-$) by targeted disruption of the gene that encodes IL-6 (*11*). These mice develop normally but fail to mount optimal responses to injury or infection. In particular, although amounts of total serum immunoglobulin are similar in $IL\text{-}6^-$ mutants and their normal ($IL\text{-}6^+$) littermates, the mutants have impaired serum IgG antibody responses to virus infection (*11*). These mice provide an ideal opportunity for assessment of the in vivo relevance of IL-6 to mucosal IgA responses (*12*). We addressed this question by determining the difference in total and antigen-specific IgA plasma cell distribution and IgA antibody production in the intestines and lungs of $IL\text{-}6^-$ and $IL\text{-}6^+$ mice immunized with protein antigen or infected with recombinant vaccinia virus (rVV).

In the absence of deliberate immunization, mutant mice had substantially fewer IgA plasma cells in their small intestines, mesenteric lymph nodes (MLN), and lungs compared to $IL\text{-}6^+$ mice (Figs. 1A and 2, A and B). In addition, IgA-positive cells stained much less intensely in the mutants. Increased numbers of IgM-positive cells were found in mutants (Fig. 1A), especially in MLN, possibly reflecting a block in the differentiation pathway toward IgA production. The paucity of IgA plasma cells in $IL\text{-}6^-$ mice was consistent with the distribution of IL-6 mRNA in intestines as determined by in situ hybridization; whereas IL-6 mRNA was broadly distributed throughout the lamina propria of $IL\text{-}6^+$ mice (Fig. 2C), no such signal was detected in $IL\text{-}6^-$ mice (Fig. 2D). The residual number of IgA plasma cells in the latter may arise from a minor subset of IgA precursor cells, which come from the peritoneal cavity rather than MALT (*13*) and which appear to develop independently of IL-6 (*14*).

We next determined the consequences of IL-6 deficiency on the development of mucosal antibody responses. The capacity of mutant mice to mount specific intestinal IgA ACC responses after local immunization with ovalbumin was grossly deficient (Fig. 1B). To study responses to virus infection, we immunized mice intranasally with rVV constructs (*15*) encoding the gene for the hemagglutinin (HA) glycoprotein of influenza virus, with or without the murine IL-6 gene (VV-HA-IL-6 or VV-HA-TK, respectively), and monitored their production of HA antibody and the number of HA-specific antibody-secreting cells (ASCs) in the lungs. Recombinant VV constructs encoding genes for foreign proteins produce these factors in a highly localized manner at sites and in amounts determined by the extent of virus replica-

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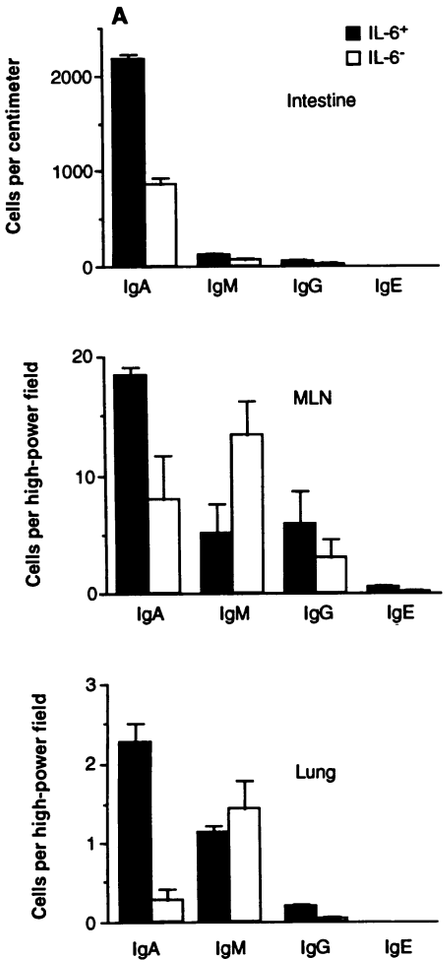

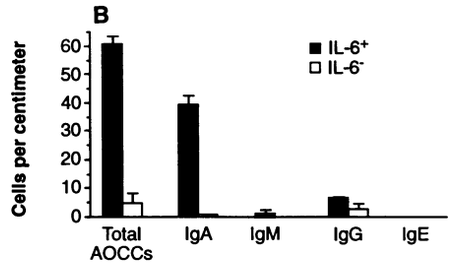

Fig. 1. (**A**) Numbers of IgA-, IgM-, IgG-, and IgE-specific plasma cells in the small intestines, mesenteric lymph nodes (MLN), and lungs of unimmunized control (IL-6$^+$) mice and IL-6$^-$ mice. Tissues were fixed in cold ethanol and embedded in paraffin (*21*). Data were obtained by immunofluorescent histology with fluorescein isothiocyanate (FITC)–conjugated or tetramethyl rhodamine isothiocyanate (TRITC)–conjugated antibodies to heavy chain reagents (*21*) and represent means of observations ± SEM in 30 to 50 randomly selected high-power (×400) fields per mouse with four mice per group. (**B**) Numbers and isotype distribution of cells containing antibodies to ovalbumin (AOCCs) in cold ethanol–fixed, paraffin-embedded small intestinal tissue from locally immunized IL-6$^+$ mice and IL-6$^-$ mice. Mice were primed intraperitoneally with ovalbumin in complete Freund's adjuvant 14 days before intraduodenal tests with ovalbumin in saline; the mice were killed 5 days later (*21*). We obtained data with double fluorochrome immunofluorescent histology (*21*) by incubating tissues sequentially with ovalbumin (1 mg/ml), TRITC-conjugated antibodies to ovalbumin, and FITC antibodies to heavy chain reagents; data represent means of observations ± SEM in 30 to 50 randomly selected high-power fields per mouse with three mice per group.

tion (*16*). Whereas IL-6$^+$ control mice given VV-HA-TK mounted strong, specific IgA and IgG responses by days 8 or 15 (Fig. 3A), mutant mice failed to develop large numbers of ASCs after virus infection (Fig. 3B). The ability of the mutants to mount sustained mucosal antibody responses was restored if they were given rVV that expressed IL-6 (Fig. 3B). This was not the case when the mutants were given rVV encoding IL-2, IL-4, or IL-5 (*17*), despite in vitro evidence that these factors also promote mucosal antibody responses (*18*). The sum of the specific ASCs against HA and the VV vector in the lungs of mutants given VV-HA-IL-6 was equal to the total number of ASCs found in these tissues (*17*). Although vector-encoded IL-6 promoted the development of lung IgA precursor cells, which are of mucosal origin, it also clearly provided proliferative signals for plasma cell precursors entering the lung from systemic immune sites, as shown by the restoration of IgG responses in mutant mice (Fig. 3B). In addition, although enzyme-linked immunosorbent assay titers of HA-specific IgA and IgG antibodies were negligible in bronchial lavage fluids from mutant mice given VV-HA-TK, strong responses, similar to those found in IL-6$^+$ mice, were detected in mutants given VV-HA-IL-6 (*17*). These findings provide evidence that IL-6 functions in the development of mucosal antibody responses to virus infection.

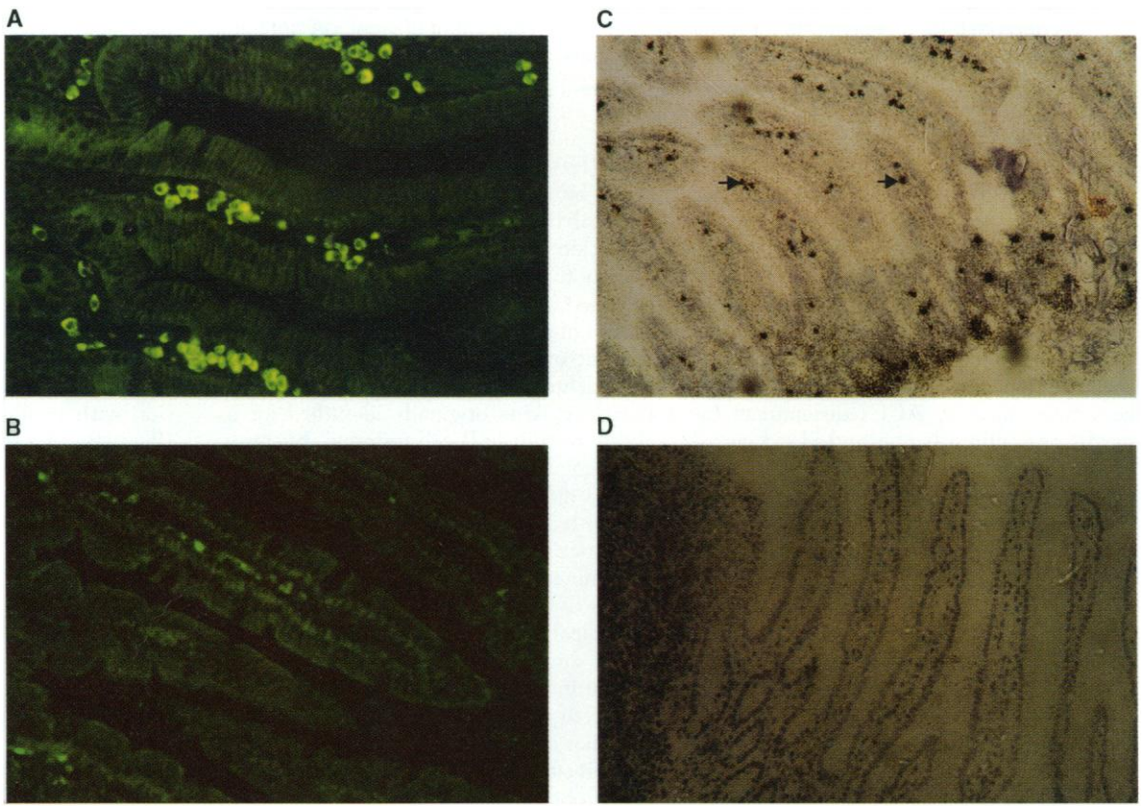

Fig. 2. Appearance of IgA plasma cells in the villus lamina propria of the small intestine in (**A**) IL-6$^+$ and (**B**) IL-6$^-$ mice. Plasma cells were detected by immunofluorescent histology with FITC-conjugated antibodies to heavy chain reagents, and tissues were prepared and treated as in Fig. 1. These are representative of many such sections taken from groups of four mice of each phenotype. (**C** and **D**) Distribution of IL-6 mRNA (arrows) in small intestinal villi of (C) IL-6$^+$ and (D) IL-6$^-$ mice. In situ hybridization was performed with ^{35}S-labeled murine IL-6 riboprobes (*10*), and tissues were counterstained with hematoxylin.

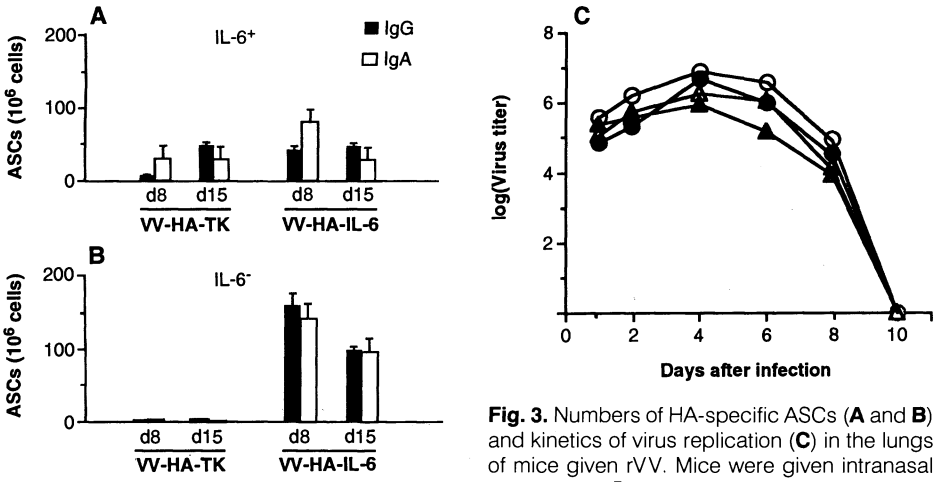

Fig. 3. Numbers of HA-specific ASCs (**A** and **B**) and kinetics of virus replication (**C**) in the lungs of mice given rVV. Mice were given intranasal inocula of 10^7 plaque-forming units of VV-HA-TK or VV-HA-IL-6 under light ether anesthesia, and their lungs were removed on the days indicated (d8, day 8; d15, day 15). Lymphoid cells were isolated from the lungs of IL-6$^+$ mice (A) and IL-6$^-$ mice (B) and assayed for IgA ASCs and IgG ASCs by ELISPOT (*19*). Values shown are mean counts ± SEM for groups of five mice and are representative of two separate experiments. The data shown in (C) are mean geometric titers of VV-HA-TK (circles) or VV-HA-IL-6 (triangles) in lung homogenates from IL-6$^+$ mice (closed symbols) or IL-6$^-$ mice (open symbols) and represent five mice per group (*19, 22*). In each case, the SEMs were less than 15%.

In addition to its ability to reconstitute responses in IL-6–deficient mice, vector-expressed IL-6 increased the number of specific IgA and IgG ASCs three- to fourfold in control mice by day 8 after infection (Fig. 3A). Similar increases were recorded in normal mice given rVV that expressed IL-5, probably through synergistic interaction of this factor with endogenous IL-6 (*19*). Large numbers of IgA ASCs, clustered at foci of rVV infection, were also seen in lung sections from normal mice given VV-HA-IL-5 or VV-HA-IL-6 (*17*). Preliminary studies also indicate that rVV-encoded IL-6 primes for enhanced IgA responses to co-expressed influenza HA antigen in the lungs upon subsequent local challenge with wild-type homologous influenza virus (*17*).

It is unlikely that the differences in the magnitude of local antibody responses between mutant and control mice were influenced by different patterns of virus growth and persistence. The VV-HA-TK and VV-HA-IL-6 constructs are attenuated and cause no obvious morbidity or mortality, and the kinetics of their replication in and clearance from the lungs were similar in IL-6$^+$ and IL-6$^-$ mutant mice (Fig. 3C). This, in turn, suggests that antibody is not relevant to the control of infection with attenuated vaccinia virus. However, IL-6$^-$ mutants, unlike control mice, cannot resolve infection with the more virulent wild-type vaccinia virus probably because of impaired cytotoxic T cell responses (*11*).

Taken together, our data demonstrate the importance of IL-6 for the development of mucosal antibody responses in vivo. This is probably because of the actions of locally produced IL-6 in promoting differentiation or proliferation of plasma cell precursors arriving at mucosal sites. In view of their deficient IgA immunity, the IL-6$^-$ mutants will be of value for studies of the relevance of mucosal IgA responses against a variety of microbial infections. The use of rVV encoding IL-6 to restore the capacity of genetically deficient mice to mount mucosal antibody responses to vaccine antigens demonstrates the effectiveness of transient, vector-directed cytokine gene therapy. The expression of cytokines and other regulatory molecules by rVV and other vectors, which mimic the physiological state more closely than alternative methods of delivering these factors, should allow manipulation of the microenvironment to favor a range of biological processes, such as the development of appropriate immune responses, in normal and genetically deficient individuals (*16, 19, 20*).

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