Critical roles of chemokine receptor CCR10 in regulating memory IgA responses in intestines

Shaomin Hu, KangKang Yang, Jie Yang, Ming Li, and Na Xiong

Center for Molecular Immunology and Infectious Diseases and Department of Veterinary and Biomedical Sciences, Pennsylvania State University, University Park, PA 16802

AUTHOR SUMMARY

The immunoglobulin A (IgA) antibody is produced by white blood cells known as plasma cells in the lamina propria, a component of the intestinal lining. From there, the antibody is secreted into the lumen, where it plays important roles in controlling commensal bacteria and neutralizing food-borne pathogens and toxins (1). Understanding the molecular mechanisms that regulate the maintenance and memory responses of intestinal IgA is important for designing better vaccines against medically important pathogens. Chemokine receptor CCR10, a protein expressed on IgA-producing cells, has been suggested to play an important role in intestinal IgA responses by regulating the intestinal migration and maintenance of the IgA-producing cells (2). However, the actual function of CCR10 in the intestinal IgA response is unclear despite a great deal of research effort. Previous studies found that interfering with CCR10 or its ligand did not impair intestinal IgA production under normal conditions or during infection (3, 4). By using a strain of mice that lacked CCR10, we found that, although IgA+ plasma cells lacking CCR10 were defective in intestinal migration, increased generation of IgA+ cells in intestinal lymphoid tissues compensated for this effect, allowing normal IgA production under healthy conditions and during the primary response to bacterial pathogen infection (Fig. P1, Upper). However, the CCR10-lacking long-lived IgA-producing plasma cells and memory B cells generated in response to the infection could not be maintained properly in the intestines. Consequently, the IgA memory responses to reinfection were severely impaired in mice lacking CCR10 (Fig. P1, Lower). These findings demonstrate critical roles for CCR10 in the intestinal IgA memory response.

To identify the functional importance of CCR10 in the intestinal IgA response, we examined the effects of removing CCR10 on the intestinal migration and maintenance of IgA+ cells under healthy conditions and during different phases of response to infection by using a strain of mice lacking CCR10 (designated CCR10-KO/EGFP-knockin mice or simply CCR10-KO mice). In these mice, the gene encoding CCR10 was replaced with the coding sequence of an EGFP, which produces a green fluorescent signal. As the on/off switches of the CCR10 gene were retained in CCR10-KO/EGFP-knockin mice, this meant that EGFP would be expressed in place of normal CCR10 expression. Therefore, EGFP served as a marker of CCR10-expressing cells in the intestines, permitting us to monitor these cells in various experiments (5). Mice carrying only one copy of this modified gene (CCR10+/-EGFP mice) will presumably be normal, as they also retain one copy of the normal CCR10 gene. However, mice carrying two copies of the modified gene (CCR10-EGFP/EGFP mice) lack CCR10 entirely.

We found that levels of total IgA+ cells and antibodies were normal in the intestines of mice lacking both copies of CCR10 (CCR10-EGFP/EGFP) compared with those of WT mice or mice lacking one copy of CCR10 (CCR10+/-EGFP) under healthy conditions. However, when the migration capacities of EGFP+ IgA+ cells from CCR10+/-EGFP and CCR10-EGFP/EGFP mice were compared by cotransferring them into WT mice, significantly fewer EGFP+IgA+ cells from the CCR10-EGFP/EGFP donor were recovered from the large and small intestines of the recipients relative to those of the CCR10+/-EGFP donor, suggesting that the CCR10-lacking IgA+ cells were defective in migration into the intestines. Hence, the apparently normal levels of intestinal IgA+ cells and antibodies in CCR10-EGFP/EGFP mice are caused by a compensation mechanism other than another receptor functioning in place of CCR10.

We found that enhanced generation of IgA+ cells in isolated lymphoid follicles (ILFs)—intestinal lymphoid tissues rich in B cells as a result of interactions with the commensal bacteria—contributes to the compensatory IgA response in mice lacking both copies of CCR10. Compared with mice that have one functional copy of CCR10 (CCR10+/-EGFP), those missing both
copies of CCR10 (CCR10\textsuperscript{EGFP/EGFP}) have twice as many ILFs in the intestines. Furthermore, a microscopic analysis detected much more (four to five fold) IgA\textsuperscript{+} cells within the ILFs of mice lacking both copies compared with mice possessing one functional copy of CCR10. These results suggest that the impaired migration of IgA\textsuperscript{+} cells into the intestines is compensated for by the enhanced production of IgA\textsuperscript{+} cells in intestine-associated lymphoid follicles such as the ILFs in mice lacking both copies of CCR10. Treatment with antibiotics stopped the enhanced generation of ILFs and IgA\textsuperscript{+} cells in mice lacking both copies of CCR10, indicating that the increased stimulation from commensal bacteria is responsible for the enhanced generation of IgA\textsuperscript{+} cells within the ILFs of these mice, which would in turn suppress the bacteria for maintaining normal intestinal environment.

As the compensation process made it difficult to dissect the role of CCR10 in the intestinal IgA response of mice lacking CCR10, we also performed competitive bone marrow transfer experiments in which similar numbers of bone marrow cells of mice lacking both copies of CCR10 and mice possessing one functional copy of CCR10 were cotransferred into WT mice, which had been lethally irradiated to kill their own bone marrow cells. Compared with IgA\textsuperscript{+} cells of the CCR10\textsuperscript{+EGFP} donor origin, IgA\textsuperscript{+} cells of the CCR10\textsuperscript{EGFP/EGFP} donor were three times less abundant in intestines but twice as abundant in intestinal lymphoid tissues such as spleens and bone marrow of the recipients, demonstrating that (i) the expression of CCR10 by IgA\textsuperscript{+} cells is important for their efficient migration and/or maintenance in the intestines, and (ii) in the absence of CCR10, the proper tissue distribution of the IgA\textsuperscript{+} cells in the intestines becomes dysregulated.

We then tested how a lack of CCR10 affected the intestinal IgA response to infection of the bacterial pathogen \textit{Citrobacter rodentium}. CCR10\textsuperscript{EGFP/EGFP} mice generally have a normal IgA response to a primary \textit{Citrobacter} infection, but the long-term maintenance of the \textit{Citrobacter}-specific IgA\textsuperscript{+} plasma cells and memory B cells in the intestines of CCR10\textsuperscript{EGFP/EGFP} mice is impaired. An ELISPOT assay detected significantly lower percentages of \textit{Citrobacter}-specific IgA antibody-secreting cells in intestinal lymphocytes isolated from CCR10\textsuperscript{EGFP/EGFP} mice than from CCR10\textsuperscript{+EGFP} controls 3 mo after their infection with and clearance of \textit{Citrobacter}. These results suggest that, although the defective intestinal migration of CCR10-deficient IgA\textsuperscript{+} cells is compensated for under healthy conditions or in the effector phase of the primary infection by the enhanced generation of IgA\textsuperscript{+} cells as a result of continuous stimulation from the commensal or pathogenic bacteria, such a mechanism did not exist to offset the impaired maintenance of the long-lived \textit{Citrobacter}-specific IgA-producing plasma cells after the bacteria are cleared. In addition, when \textit{Citrobacter}-specific IgA\textsuperscript{+} memory B cells were assessed in the CCR10\textsuperscript{EGFP/EGFP} and CCR10\textsuperscript{+EGFP} mice long after the bacterial clearance, their numbers were also significantly lower in intestines of the CCR10\textsuperscript{EGFP/EGFP} mice. The numbers of long-lived \textit{Citrobacter}-specific IgA\textsuperscript{+} plasma and memory B cells in the internal tissues of the infected CCR10\textsuperscript{EGFP/EGFP} mice were increased or not changed, indicating that the CCR10 KO specifically impaired maintenance of these cells in the intestines.

The impaired intestinal maintenance of the \textit{Citrobacter}-specific IgA\textsuperscript{+} plasma cells and memory B cells affects the memory response in CCR10\textsuperscript{EGFP/EGFP} mice. In striking contrast to the generally normal primary IgA response, the production of IgA in memory response to \textit{Citrobacter} reinfection in the previously infected CCR10\textsuperscript{EGFP/EGFP} mice was severely impaired. The reinfected CCR10\textsuperscript{EGFP/EGFP} mice had slower appearance and lower levels of the \textit{Citrobacter}-specific IgA antibodies in the feces than the CCR10\textsuperscript{+EGFP} controls did. In addition, the levels of the \textit{Citrobacter}-specific IgA in the feces of CCR10\textsuperscript{EGFP/EGFP} mice decreased rapidly after reaching the peak level, whereas the CCR10\textsuperscript{+EGFP} mice maintained a high level of the \textit{Citrobacter}-specific IgA for a long time. On the whole, the IgA production pattern in the reinfected CCR10\textsuperscript{EGFP/EGFP} mice was not significantly different from that of the primary response, indicating that they are almost devoid of the IgA memory. By using the ELISPOT assay, we confirmed that the severely impaired production of fecal \textit{Citrobacter}-specific IgA antibodies in the reinfected CCR10\textsuperscript{EGFP/EGFP} mice was caused by the reduced numbers of \textit{Citrobacter}-specific IgA-secreting cells in the intestines. At day 14 after the reinfection, the numbers of \textit{Citrobacter}-specific IgA-secreting cells in large intestines of CCR10\textsuperscript{EGFP/EGFP} mice were drastically (15-fold) lower than those in the CCR10\textsuperscript{+EGFP} controls, whereas the numbers were also significantly (threefold) reduced in the small intestines. These results also suggest that CCR10 KO impairs the memory responses in the small and large intestines to different extents, likely because of differential expression of other chemokine molecules involved in the migration and maintenance of IgA\textsuperscript{+} cells in these tissues, such as the CCR9 ligand CCL25 that is expressed only in the small intestine.

In summary, our findings demonstrate that CCR10 plays a critical role in migration and the long-term presence of IgA plasma cells and memory B cells in intestines for efficient IgA maintenance and memory response. Considering that immune activity against infectious pathogens at their entry sites is critical in preventing the establishment of the infection, our findings provide a mechanistic basis for manipulating the CCR10/ligand axis in designing better vaccines against the pathogens that infect intestines and other mucosal sites such as lungs and reproductive tracts, where IgA also plays an important role. Our findings also helped to elucidate the processes involved in the maintenance of normal intestinal environment and functional mechanisms of CCR10 in such processes.