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Enhancing immune responses to limit chronic immune activation during SIV

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The persistent immune activation that is typical of HIV-1 and SIV infection results in exhaustion and dysfunction of T and B cells; in T cells, this is marked by increased expression and signaling through the inhibitory receptor programmed death–1 (PD-1). Targeting this exhaustion pathway could result in improved antiviral immune responses, but there have been concerns that it would also lead to increased inflammation and immunopathology. In this issue of the *JCI*, Dyavar Shetty et al. demonstrate that blocking PD-1 actually reduced proinflammatory responses and improved immunity in the gut of SIV-infected rhesus macaques, suggesting that this might have therapeutic potential to prevent opportunistic infections in HIV-infected patients.

Persistent immune activation is a hallmark of HIV-1 infection in humans and pathogenic SIV infection in rhesus macaques (RMs). HIV and SIV infections induce robust, generalized inflammatory responses that begin during acute infection and lead to pathological systemic immune activation, marked by microbial translocation, fibrotic damage of lymphoid tissues, and CD4+ T cell loss, processes that contribute to pathogenesis and disease progression. Pathological chronic infections with primate immunodeficiency viruses are further characterized by exhausted and dysfunctional T and B cell antiviral immune responses (1, 2) that contribute to the inability of the host to eliminate the persistent viral pathogen. The exhaustion of virus-specific T cells in chronically persistent virus infections is not unique to lentivirus infections, as this phenomenon was first demonstrated in the murine lymphocytic choriomeningitis virus (LCMV) model (3, 4), and subsequently virus-specific T cell exhaustion was demonstrated in HIV, HBV, and HCV infections of humans (5–8) and SIV infection of RMs (9, 10).

Can preventing exhaustion improve immune responses?

Exhausted T cells are characterized by increased expression of the inhibitory receptor programmed death–1 (PD-1), and in the LCMV model, blockade of the PD-1 pathway restored virus-specific T cell function, leading to viral clearance (11). While blockade of PD-1 enhances HIV- and SIV-specific T cell cytokine production and proliferation in vitro (9, 10), initially there was skepticism that this method would restore immunological function in the pathogenic RM SIV model in vivo (12). This skepticism was based on the premise that if in vivo blockade of the PD-1 inhibitory receptor in chronic SIV infection could result in enhanced antiviral T and B cell responses, it could also potentially exacerbate bystander T cell activation in an already persistent inflammatory setting, potentially leading to accelerated disease progression.

Studies using anti–PD-1 antibody in chronically SIV-infected RMs have produced mixed results. Finnefrock et al. showed that a single injection of anti–PD-1 antibody produced no change in SIV-specific T cell function or numbers, with a transient increase in viral loads (13), while Velu et al. demonstrated that four repeated administrations of anti–PD-1 antibody resulted in enhanced SIV-specific immunity and prolonged survival (14). In this issue of the *JCI*, this latter group expands their analysis of anti–PD-1–treated RMs in an attempt to understand the putative mechanism for prolonged survival (15).
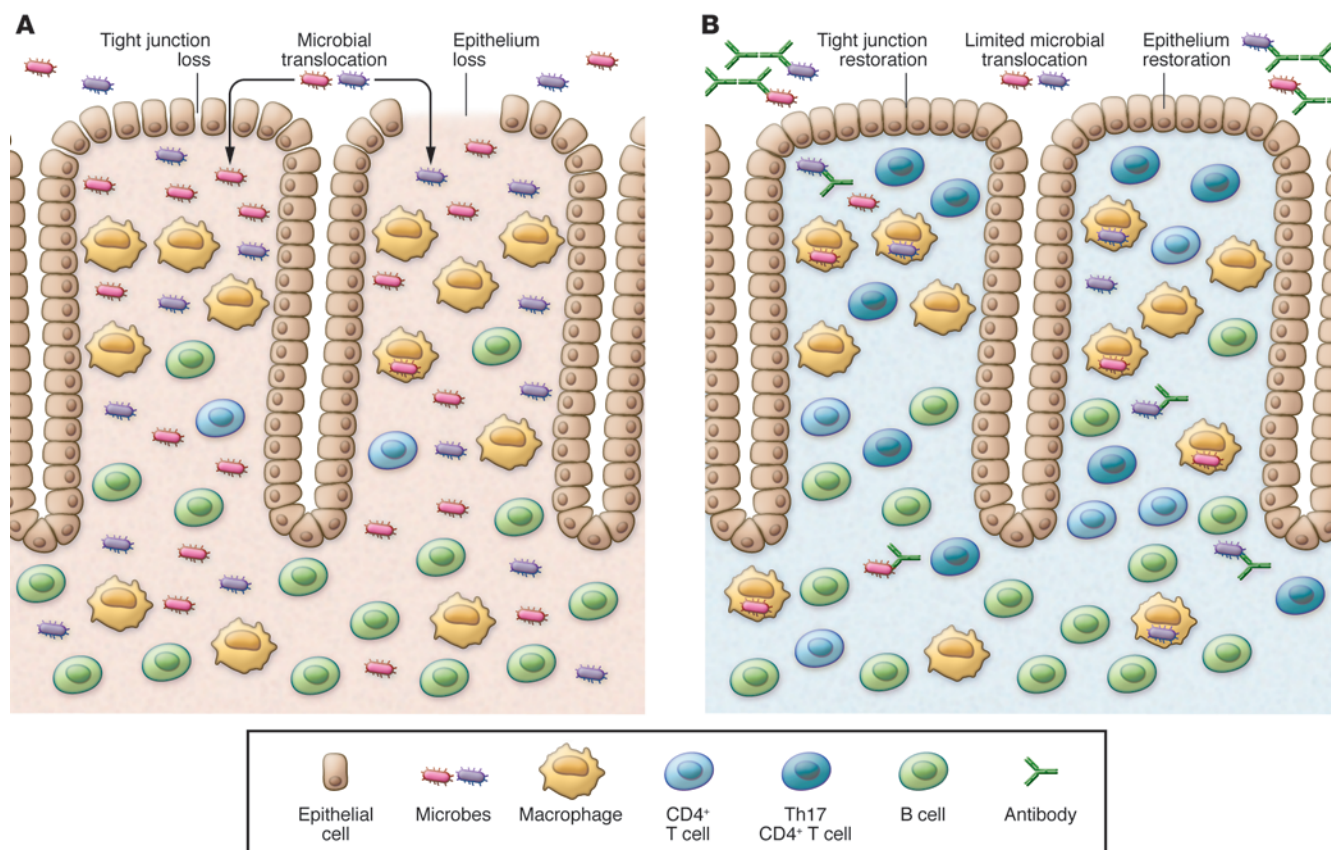
Figure 1
Simplified model of intestinal immunopathology and restoration with anti–PD-1 antibody treatment in SIV infection. (**A**) SIV infection results in a chronic proinflammatory environment, leading to exhausted/dysfunctional T and B cells, increased epithelial turnover, multifocal epithelial breaches and loss, microbial translocation, dramatically elevated ISGs, and macrophage dysfunction including inadequate microbial constituent clearance. (**B**) Treatment of SIV-infected RMs with anti–PD-1 antibody results in a reduced proinflammatory environment, significantly increased SIV-specific and microbial T and B cell function, increased epithelial barrier maintenance and function with increased tight junction expression, reduced microbial translocation, reduced ISG expression, and potentially restored macrophage function including improved microbial constituent clearance. However, the mechanisms that underlie the effects of anti–PD-1 antibody action are incompletely understood.

They demonstrate that although anti–PD-1 antibody treatment in SIV-infected RMs was initially intended to enhance T and B cell SIV responses as a means of viral control, it resulted in a significant reduction in proinflammatory responses, leading to enhanced gut immunity, reduced microbial translocation, restoration of the integrity of the gastrointestinal (GI) epithelial barrier, limited opportunistic infections, and significantly prolonged survival compared with controls (Figure 1).

That anti–PD-1 antibody treatment would result in reduced proinflammatory responses rather than enhancing T and B cell effector functions and proliferative capacity seems somewhat paradoxical. Nevertheless, anti–PD-1 antibody treatment of chronically SIV-infected RMs resulted in significant downregulation of IFN stimulatory genes (ISGs) (15), which are elevated in association with chronic immune activation and correlate with faster disease progression in SIV-infected RMs (16, 17).

Gut reaction to viral infection

The reduction in ISGs becomes more understandable when seen in the context of our current understanding of the pathogenesis of immune activation in pathogenic SIV infection. Anti–PD-1 treatment resulted in a reduced level of microbial translocation, demonstrated by reduced plasma LPS levels. The effect of anti–PD-1 antibody treatment on ISG expression and microbial translocation was independent of gut viral burden, as anti–PD-1–treated and control RMs had equivalent levels of SIV viral RNA in colonic tissue samples (15). Strong downregulation of type I IFN responses with anti–PD-1 antibody treat-

ment in the absence of any effect on gut viral burden reinforces the hypothesis that SIV infection disrupts the GI epithelial barrier, resulting in microbial translocation that in turn helps drive immune activation (18). GI epithelial barrier damage and associated microbial translocation are spatially associated with type I IFN expression within the lamina propria and lymphoid tissues of chronically SIV-infected RMs in a manner not strictly linked to local viral replication, supporting the hypothesis that microbial products — rather than local viral replication — stimulate innate inflammatory responses within the damaged gut (18).

Intriguingly, Dyavar Shetty and colleagues found that anti–PD-1 antibody treatment appeared to help restore GI epithelial barrier integrity, resulting in increased expression in the gut tissue of

genes associated with tight junctions, along with reduced microbial translocation and type I IFN responses both locally and systemically (15). It is unclear whether the effect on GI epithelial cells in anti–PD-1 antibody–treated animals is due to direct effects on their survival (as these cells do express the PD-L1 ligand) or indirect effects from reduced inflammation and ISG expression due to better clearance of microbial constituents and restoration of the GI epithelial barrier. Regardless of the mechanism, anti–PD-1 antibody treatment significantly reduced microbial translocation and associated ISG expression, changes that correlated with improved survival for anti–PD-1–treated animals (15).

Making connections

While the effect of anti–PD-1 antibody treatment on GI epithelial cell integrity may have been unexpected, during chronic lentiviral infections the exhaustion of T and B cells may be partially independent of antigenic specificity, reflecting instead the cumulative effects of the overall local inflammatory milieu in tissue sites. Thus, one potential factor contributing to sustained microbial translocation in chronic HIV and SIV infections may be exhaustion of microbe-specific T and B cells within the gut and dysfunctions in resident gut macrophages, collectively limiting microbial clearance. Thus, anti–PD-1 antibody treatment may improve not only SIV-specific T and B cell responses, but also T and B cell responses to common gut-resident bacteria. Indeed, Dyavar Shetty et al. found that anti–PD-1 antibody treatment had an important effect on adaptive immune responses to common gut-resident bacteria (15). Specifically, treatment significantly increased serum *Campylobacter*-specific antibody levels and *Campylobacter*-specific and *Salmonella*-specific peripheral blood CD8^{+} T cell responses in most of the anti–PD-1–treated RMs. In contrast, in control RMs the CD8^{+} T cell responses to these same gut-resident bacteria decreased over time, despite ongoing exposure to gut microbes, as reflected in evidence of microbial translocation (15).

In addition to the direct impact of anti–PD-1 treatment on restoring T and B cell function, several recent studies have implicated PD-1 expression and ligation on macrophages in either the development of macrophage dysfunction or CD4^{+} T cell dysfunction (19, 20), suggesting another mechanism by which the immunoregulatory effects of PD-1 expression extend beyond direct PD-1–mediated T and B cell exhaustion. Because a recent study suggested that there may be a dysfunction in the ability of lamina propria macrophages to effectively phagocytose translocated microbial constituents in SIV-infected RMs (18), it will be important to determine whether anti–PD-1 antibody treatment results in improved function and microbial clearance by gut macrophages.

Since HIV infection is associated with exhausted/dysfunctional T and B cell responses to the virus, and presumably to other pathogens, an important treatment outcome in HIV patient care would be improved immune responses to these pathogens, particularly the etiologic agents of opportunistic infections. However, it is important to note that deletion of pathogen-specific T cells and not just their dysfunction can contribute to the development of opportunistic infections (21). Dyavar Shetty et al. observed a marked reduction in putative opportunistic infections in anti–PD-1 antibody–treated chronically SIV-infected RMs despite continued high levels of viral replication (15), suggesting that even in the absence of antiretroviral therapy, inhibiting the PD-1 signaling pathway may be able to overcome T and B cell exhaustion/dysfunction and thereby improve clinical outcome independent of effects on viral load or CD4^{+} T cell count (14), two commonly used clinical indicators of disease progression (21).

Perspectives

While the results reported by Dyavar Shetty and colleagues are provocative and encouraging, their study leaves many important questions unanswered. For example, is the effect of anti–PD-1 antibody treatment sufficient to confer a phenotype similar to that seen in non-pathogenic, non-progressive SIV infections? What effect does anti–PD-1 antibody treatment have on generalized T and B cell activation and proliferation during pathogenic HIV and SIV infections in the absence of attenuated viral replication? What global effect does anti–PD-1 treatment have on the cytokine milieu within lymphoid and gut tissue compartments, and how does this impact tissue architecture regeneration? What impact does anti–PD-1 treatment have on macrophage survival and function, particularly within the GI tract? Would longer anti–PD-1 antibody treatment or additional treatment cycles result in sustained reductions in microbial translocation, low levels of ISGs, and improved GI tract CD4^{+} T cell (particularly Th17 cell) recovery? Does blockade of PD-1 signaling in GI tract epithelial cells directly result in improved enterocyte function and regenerative capacity?

Though these and other important questions will need to be addressed, the unanticipated benefits following anti–PD1 treatment underscore the complexity of the immunopathology of AIDS virus infections and provide support for considering evaluation of anti–PD-1 antibody treatment in small, targeted human clinical trials aimed at decreasing immune activation and enhancing viral specific immunity.

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