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### **Commentary**

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# B cells join T cell clusters in the host response to recurrent herpes simplex virus 2 infection

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**Recurrent genital herpes lesions are infiltrated by various leukocytes, yet the role of B cell subsets in this process is unknown. In this issue of the JCI, Ford et al. describe the presence and antibody-secreting role of local B cell populations in herpes simplex virus 2 (HSV-2) recurrent lesions. The authors analyzed a comprehensive array of sequential skin biopsy specimens from HSV-2-infected patients over time and at various stages of infection. Using immunofluorescence and in situ hybridization, the authors show the presence of rare IgD<sup>+</sup> naive B cells and IgG-expressing antibody-secreting cells (ASCs) in recurrent HSV-2 lesions embedded in CD4<sup>+</sup> T cell-rich dermal immune infiltrates, levels of which transiently increase during lesion reactivation and healing. Notably, local increases in HSV-2-specific antibodies in recurrent lesions were detected, whereas serum HSV-2 antibody levels remained stable. Future research is needed to understand the precise role of these tissue-visiting B cells in disease resolution.**

failure of circulating neutralizing antibodies to confer protection at the mucosa. First, circulating antibodies have limited capacity to cross the intact vaginal epithelial barrier to block viral invasion (8); and second, these antibodies are circumvented by the viral glycoproteins gE, which serves as a decoy Fc receptor, and glycoprotein C (gC), which abrogates complement activation (9). However, our understanding of humoral immunity in the context of human HSV-2 prevention and disease is incomplete; additional studies of the B cell lineage, antibodies, and their role in controlling HSV-2 infection are critical for informing future vaccine design.

## B cells and antibody responses to genital herpes

The role of humoral immunity in HSV-2 in humans is unclear; however, preclinical models suggest that B cells and antibody responses do aid in the immune response to HSV-2 genital disease to some degree. The antigen presentation function of B cells as well as natural antibodies have a limited protective role in the primary infection response to HSV-2 (10, 11). HSV-2-immune, B cell-deficient mice only have mildly elevated vaginal viral load but are protected from disease and death after HSV-2 challenge (11, 12). In addition, systemically introduced HSV-2-specific antibodies are unable to prevent HSV-2 infection (12), likely due to the limited access of antibodies across the vaginal epithelial barrier (8, 13, 14). This raises the question of whether circulating antibodies alone would have any role in preventing HSV-2 infection or facilitating recurrent lesion resolution.

To answer this question, we must consider the role of T cell-mediated immunity in cutaneous and genital HSV-2 infections (4, 15, 16). Existing studies in mouse models of genital herpes infection support a crucial role for tissue-resident local T cells in conferring protection against genital HSV-2 challenge (17–19). Circulating

## HSV-2 and human disease

Herpes simplex virus 2 (HSV-2) is a major cause of genital ulcers worldwide and among the most common sexually transmitted infections (STIs), with seroprevalence studies demonstrating infection in more than 15% of the US population (1–3). Infection is more common in women (21%) and in non-Hispanic Black individuals (39%; ref. 1). Though most commonly self-limited in the immunocompetent host, infections are lifelong, and in many patients, periodic recurrences are a substantial source of morbidity, with an additional psychosocial burden given stigmatization of genital disease. Moreover, HSV-2 infection is a cofactor increasing the risk of other STIs, including HIV (3, 4). In immunocompro-

mised populations, more-severe systemic presentations may occur, including liver, lung, and CNS disease. HSV infection in the neonatal period can be devastating, with high mortality and neurologic sequelae (3, 4).

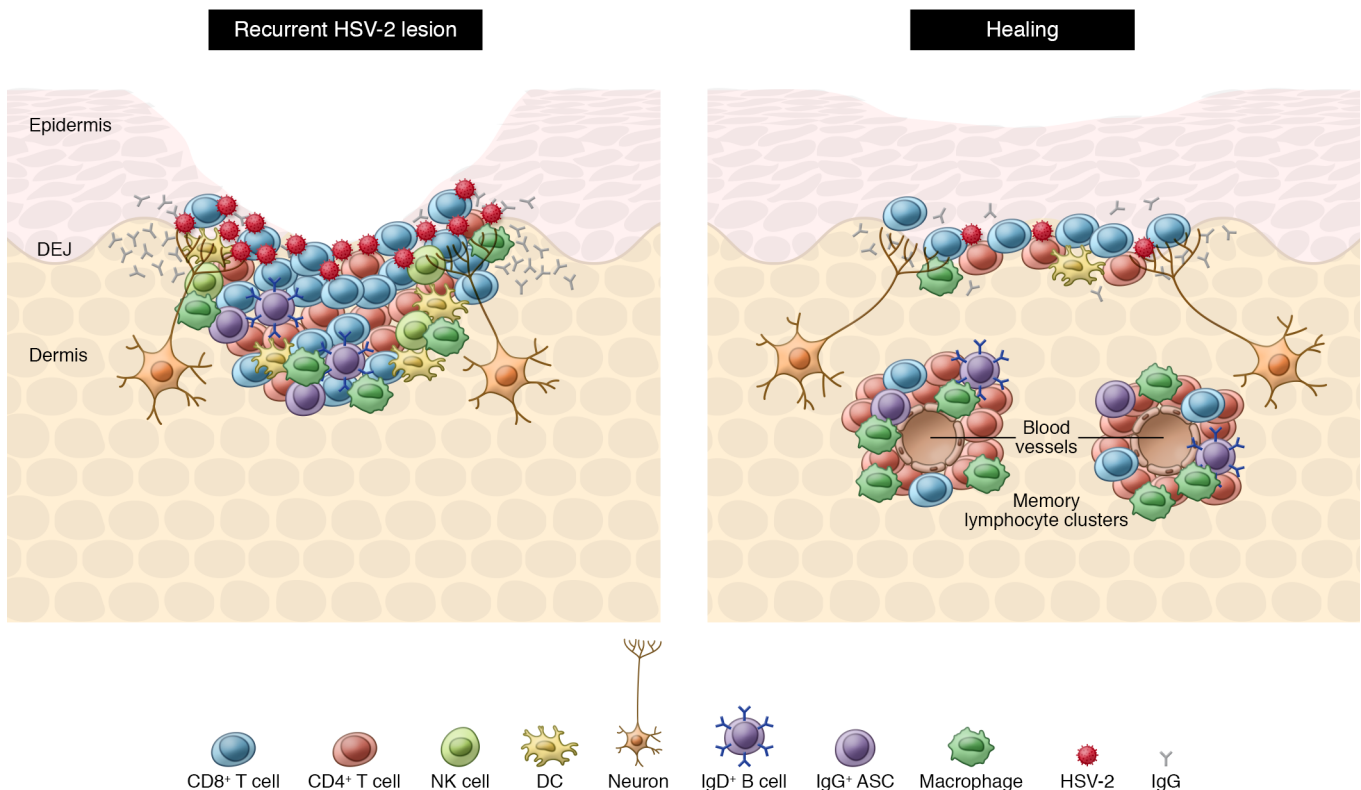
Despite substantial vaccine efforts extending into decades, no vaccine approach has proven effective in preventing HSV-2 in randomized human trials (5). Most recently, an HSV-2 glycoprotein D-based recombinant protein vaccine showed initial promise in preventing genital HSV-2 infection (6), though it failed to demonstrate protection in a broader study (7). Notably, potent neutralizing antibodies developed in vaccinated patients yet failed to effectively prevent HSV-2 infection. There are likely two reasons for this

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**Figure 1. Model of cutaneous and mucosal immune responses to HSV-2 reactivation.** In recurrent infections, clusters of antiviral CD4<sup>+</sup> and CD8<sup>+</sup> T cells form in the dermis and submucosa, along with dendritic cells and macrophages. Interferon production from T cells helps resolve the viral insult. Naive B cells and ASCs are recruited to these dermal clusters. The latter secrete antibodies that may contribute to blockade of viral spread. During healing, MLCs form. As lesions heal over time, T cell density decreases (though T<sub>RM</sub>s remain), and B cell populations are no longer, or rarely, observed. DEJ, dermal-epidermal junction.

memory T cells enter and reside in many organs but fail to establish residency in certain anatomic locations, including the skin and vagina, in the absence of local stimuli (15). Both CD8<sup>+</sup> and CD4<sup>+</sup> tissue-resident memory T cells (T<sub>RM</sub>s) have important functional roles in the local antiviral response. CD8<sup>+</sup> T<sub>RM</sub>s secrete cytokines and provide superior effector responses (17, 19, 20), whereas CD4<sup>+</sup> T<sub>RM</sub>s secrete tonic IFN- $\gamma$  and establish local memory lymphocyte clusters (MLCs) with macrophages, which are required for protection following a secondary HSV-2 challenge (18). The importance of tissue-resident T cells in human genital HSV-2 has been shown in multiple studies (21–25). HSV-2 lesion resolution correlates with a strong lymphocyte-derived effector response (22, 24). Persistent, localized, HSV-2-specific CD3<sup>+</sup> T lymphocytes are more than 90% CD4<sup>+</sup> in the genital tract of female HSV-2-infected patients (23). Clusters of predominantly CD4<sup>+</sup> T cells typically occupy perivascular locations in

the upper dermis and HSV-2 lesional skin (4), resembling MLCs seen in mouse models of genital HSV-2 infection (18). These MLCs have been characterized primarily for T cells and their effector functions, but also appear to act as an outpost for recruiting circulating virus-specific B cells during secondary infection.

In a mouse model of genital herpes, intravaginally immunized mice recruited circulating memory B cells and secreted antibodies into the vaginal mucosa after secondary challenge. Conversely, mice that received subcutaneous HSV-2 immunization failed to recruit circulating memory B cells and secrete mucosal antibodies (8). This local antibody-secreting cell (ASC) recruitment to the mucosa was dependent on the CXCR3 chemokines induced by local CD4<sup>+</sup> T<sub>RM</sub>s. Notably, no tissue-resident memory B cells or plasma cells were found 5 weeks after primary genital infection with HSV-2. In contrast, intravenous immunization of macaques with simian immunodeficiency virus pro-

moted enrichment of local plasma cells and antibody secretion in the female reproductive mucosal epithelium, which correlated with protection from disease (26). In this issue of the *JCI*, Ford et al. examined what happens in genital herpes lesions in humans (27).

### B cell recruitment to recurrent herpes lesions in humans

Ford et al. examined longitudinal biopsy specimens of HSV-2-infected skin in 16 participants (14 women and 2 men) with a median age of 50 years (27). Biopsies were performed on clinically active HSV-2 lesions; and healed lesions at 10–14 days after active infection and at 2, 4, and 8 weeks thereafter; and on nonlesional skin as controls. B cells, identified by CD20 and CD79b, were seen in at least two biopsy specimens from each study participant; they were generally more common during active lesions and early healing, yet rarely seen in control biopsies (noted in 3 of 16). Two major populations of B cells were iden-

tified: naive IgD<sup>+</sup> B cells and IgG<sup>+</sup> ASCs. The highest naive IgD<sup>+</sup> B cell and IgG<sup>+</sup> ASC densities were observed at the lesional and healing time points (within the first two weeks), which coincided with the time points of maximal T cell density. These B cell populations were characteristically identified within clusters with T cells, suggesting a possible shared mechanism for recruitment and cooperation. The authors also measured tissue HSV-2-specific antibodies and detected increases compared with antibodies against control antigens, though the time points of maximal density varied across patients and across specimens taken at time of lesion, time of healing, and two to eight weeks after healing. The highest IgG antibody density within lesions was coincident with abundant HSV-2 antigen, suggesting an active role of tissue antibody in the local HSV-2 host response. These studies are the first to our knowledge to identify, characterize, and functionally implicate B cells within the local human recurrent HSV-2 lesions. Given that serum HSV-2 antibody responses remained stable over time, the work of Ford et al. and others highlights the importance of studying humoral host immunity in the mucosal and cutaneous tissues (27).

## Implications and future directions

Through the use of their unique biospecimens and well-executed microscopy-based experiments, Ford and colleagues provide fresh insights into the B cell lineage and cutaneous HSV-2 infection (27). The identification of antigen-inexperienced B cells, as well as ASCs, suggests a more complex role for B cells in the response to local HSV-2 reactivation. Though naive B cells are not expected to play a substantial antigen-presenting role (28), their localization within T cell clusters suggests that the naive B cells may be a source of factors that influence the local inflammatory response to virus. Based on preclinical studies, the enrichment of tissue-localized ASCs portends increased mucosal antibody secretion and protection (8, 26). Though tissue-localized ASCs are rare, identifying these cells provides another clue to strategies that may boost immunity to HSV and viral infections, including enhancing ASC recruitment to infection sites to increase local antibody production.

Importantly, though, questions remain about HSV-2 skin and mucosal immunity. What is the function of naive B cells in the lymphocyte clusters in recurrent lesions? How are these B cells recruited? Do antiviral antibodies produced by the local ASC result in better viral control? Does antigen presentation by virus-specific B cells promote stimulation of CD4<sup>+</sup> T cells, which are the major effector cells that control HSV-2 infection (23)? By virtue of their sample population, the patients included in this study were those who failed to mount an effective immune response to prevent HSV-2 reactivation. While we do have a clearer picture of the immune milieu in human HSV-2 lesions over time, these data do not necessarily inform us of presumptively more-effective immune responses that may prevent infection; though, obviously, such data would be far more difficult to acquire in humans, given these patients would not manifest clinically overt disease.

Incorporating these data with existing studies of human (4, 21–25) and preclinical HSV-2 models (8, 10, 18), we can better construct a hypothesized model of local skin- and mucosa-directed HSV immunity (Figure 1). Both CD8<sup>+</sup> and CD4<sup>+</sup> T<sub>RM</sub>s have essential roles in the coordination of local responses, with CD8<sup>+</sup> T<sub>RM</sub>s providing strong cytotoxic responses and CD4<sup>+</sup> T<sub>RM</sub>s establishing MLCs that provide a cytokine-rich dermal niche critical for recruiting other cells supporting a local immune response. These additional cell types include CCL5-secreting macrophages as well as B cell populations and ASCs, which secrete antibodies locally that aid in clearing pathogens. With a more comprehensive view of this picture as well as the immunization strategies that maximally promote these localized responses, we will be one step closer to developing a vaccine and therapy that provide protection from HSV infection and/or reactivation.

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