supported the striking similarities of the mathematical simulations with the experimentally observed changes in Sox9 periodicity and digit patterns.

Together with the previous study by Sheth *et al.* (*7*), the analysis by Raspopovic *et al.* provides strong experimental support for a Turing-type mechanism controlling the emergence of the periodic pattern of the Sox9-positive digit primordia in mouse limb buds. Identification of the BMP and WNT signaling pathways suggests that it may be possible to genetically manipulate the BSW Turing network. As the antero-posterior (AP) polarity of the limb bud mesenchyme and digit progenitors are specified much earlier by graded SHH signaling (see the figure, left) (*10*), it will be important to understand which mechanism links this early AP axis polarization to the BSW Turing network that controls the stereotypic pattern of the digit condensations during autopod development. These early and late patterning systems can be genetically uncoupled, as digit condensations can occur in the absence of the SHH signaling system and disruption of the Turing network results in loss of all digit primordia without effects on early AP patterning (*4*, *11*, *12*). During evolution of tetrapod limbs, the two mechanisms must have become interlinked as digit numbers were reduced from rudimentary polydactyly (six or more digits) to pentadactyly (five digits with distinct AP identities). One likely link involves the SHH-mediated regulation of *Hoxd* expression in limb buds. This in turn modulates the BSW Turing network and, in concert with FGF signaling, confers robustness on the periodic expression pattern of *Sox9* (*4*, *7*).

Now that we know that a Turing-type mechanism controls the periodicity of digits, the next challenge will be to determine if Turing-type mechanisms are involved in coordinating the formation of digits with the patterning of other limb tissues such as tendons, ligaments, and the musculoskeletal anatomy. ■

PHOTO: JANICE MURRAY AND MAIZELS LABORATORY/UNIVERSITY OF EDINBURGH

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IMMUNOLOGY

How helminths go viral

Cellular signals during helminth infections can skew the immune response to favor viral spreading

By **Rick M Maizels**[1] *and* **William C Gause**[2]

Research into infectious diseases is generally highly reductionist, focusing on the disease-causing agent while meticulously excluding extraneous factors, such as unrelated pathogens. But the real world is quite different, with multiple concurrent microorganisms (viruses, bacteria) and macro-organisms (parasites), each with differing dynamics and impacts on the host (*1*). Many of these agents are relatively neglected, especially those such as the helminth worms (see the photo). They also predominantly affect people in low-income tropical environments and influence susceptibility to a range of other infectious diseases (*2*). On pages 573 and 578 of this issue, Reese *et al.* (*3*) and Osborne *et al.* (*4*), respectively, provide fine detail on how helminth worms can substantially enhance and reactivate viral infection, with major health implications for tropical medicine.

Heligmosomoides polygyrus

Reese *et al.* found that a latent murine herpesvirus infection in macrophages was reactivated by either of two helminth worm species. Because helminths are strong activators of T helper cell 2 (T_H2) (*5*), the authors propose a skewing of immunity toward the key T_H2 cytokines interleukin-4 (IL-4) and IL-13, and away from the antiviral cytokine interferon-γ (IFN-γ). T_H2 environments also stimulate the "alternative activation" of macrophages through the IL-4 receptor toward an "M2" state (*5*, *6*). Notably, in helminth-infected mice, many virally infected macrophages expressed the enzyme arginase, a marker of M2 macrophages that is expressed in response to IL-4 receptor activation. Exposure to IL-4 greatly increased viral replication in macrophages in vitro. In addition, either IL-4 or IL-13 (both bind to the IL-4 receptor) replicated the effects of worm infection, but not in mice lacking signal transducer and activator of transcription 6 (STAT6), a signaling molecule that is activated by the IL-4 receptor. Moreover, IL-5, a cytokine that is released by T_H2 cells during helminth infection but does not activate STAT6, failed to reawaken the virus from latency. The ability of IL-4 to promote viral growth was directly antagonized by IFN-γ, however.

How does herpesvirus break out of latency in response to IL-4? Reese *et al.* show that in infected macrophages, STAT6 binds to the promoter of *gene50*, a viral gene associated with exit from latency (see the figure). This was the case both for murine γ-herpesvirus and for the related human Kaposi's sarcoma–associated herpesvirus. This surprising degree of evolutionary conservation presumably reflects an adaptation that permits the viruses to sense the immunological status of their host. Thus, when the cytokine environment is dominated by IFN-γ (which activates a spectrum of antiviral mechanisms), the virus remains latent; but if the coast is clear and IL-4 prevails, the virus exits the latent state.

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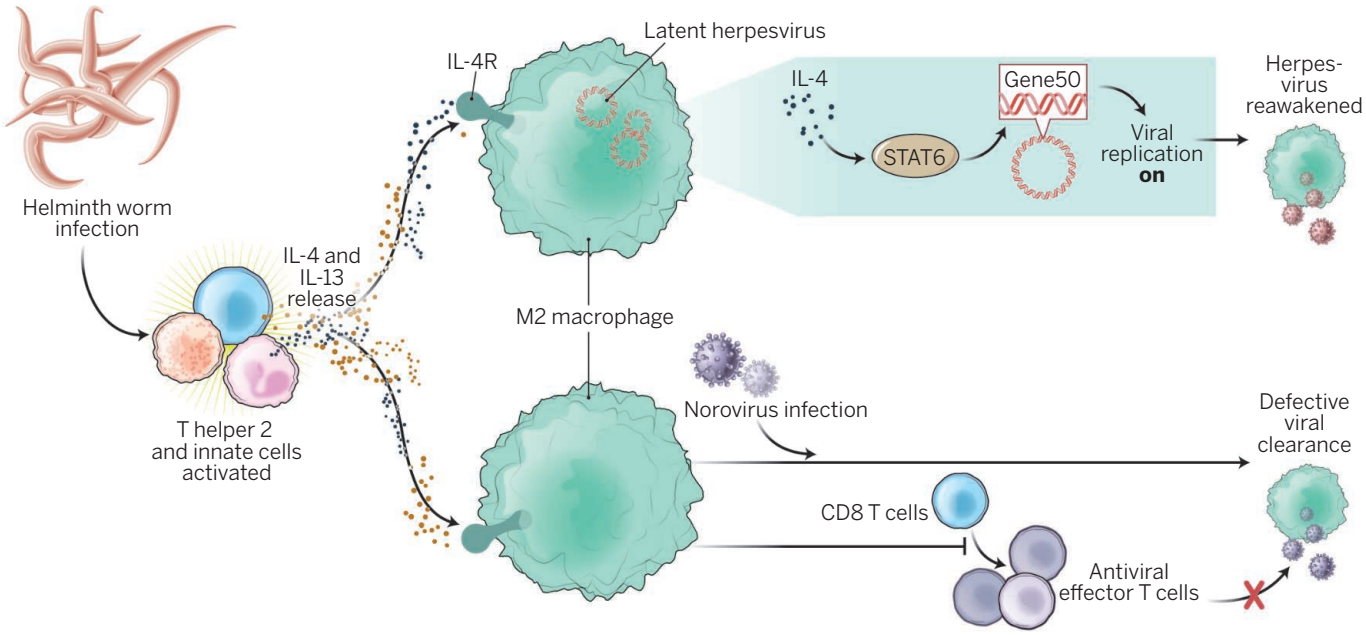

Altered immunity. Helminth infection activates T_H2 cells to release IL-4 and IL-13, both of which ligate the IL-4 receptor (IL-4R) on M2 macrophages. In M2 macrophages harboring latent herpesvirus, the IL-4R activates host cell STAT6, which then acts directly on the key viral gene that initiates viral replication. In another scenario, M2 macrophages activated by IL-4 and/or IL-13 directly inhibit the production of virus-specific T cells. Thus, subsequent infection by a virus (norovirus shown) is not controlled.

The study of Osborne *et al.* follows a similar storyline of helminth exacerbation of infections, but with a quite different plot. The authors also linked greater viral growth to the alternatively activated M2 phenotype of the host macrophage. Moreover, during helminth infections, a range of T cell responses to viral infection are severely down-regulated. Although helminth-associated T cell hyporesponsiveness develops through multiple routes (*7*), in the setting of a viral infection (in this case, by norovirus), Osborne *et al.* connect the M2 macrophages to inhibition of the T cell proliferation. In the absence of STAT6, T cell functions were rescued and viral loads diminished, whereas exposing macrophages to IL-4 increased viral production in the cells. Mice receiving IL-4–treated macrophages showed reduced T cell responses alongside higher viral titers. This regulatory function of M2 macrophages in this setting was further linked to a prominent secreted product, Ym-1, whereas inhibition of other macrophage products, including arginase, were not found to differ in helminth promotion of infection.

Osborne *et al.* also addressed the question of whether the effect of helminths on virus load was mediated by direct modulation of the host immune system, or was acting indirectly through changes to the commensal intestinal bacterial load. Changes in microbiota populations in mice following helminth infections have been observed (*8*, *9*), but Osborne *et al.* found that even in germ-free mice, the introduction of a helminth infection exerted the same depressive effect on immunity to the virus. It will be of interest to examine the extent to which helminth-induced changes in the intestinal microbiome may affect host responses to other viruses and other types of pathogens.

Beyond the similarities, the studies of Reese *et al.* and Osborne *et al.* have fascinating contrasts. In the herpesvirus system, an exquisite adaptation of the viral genome is a promoter sequence that recognizes STAT6 as well as the prevailing T_H2 environment of a helminth-infected host. Although evolutionarily conserved, this mechanism is operative only in this set of related viruses. However, helminth infections also drive alternative activation of macrophages through STAT6, generating virus-nonspecific pathways that inhibit the generic T cell response—a mechanism that would impede immunity to all viral challenges. These quite different effects of helminth infection on viral immunity are likelzy the tip of the iceberg in terms of the range of mechanisms through which helminths influence immune responses to microbial pathogens. Increasing evidence suggests that these dynamic interactions resulting from helminth coinfections may have substantial effects on susceptibility to global microbial pathogens. In particular, recent studies suggest that urogenital schistosomiasis may increase susceptibility of African women to HIV (*10*). However, it should be kept in mind that the type 2 immune response stimulated by helminths may also mitigate tissue damage during microbial infections by reducing harmful inflammation and directly enhancing wound repair (*11*).

The findings of Reese *et al.* and Osborne *et al.* deepen our perspective of the complexity of infectious diseases, given that multiple colonization is ubiquitous in nature and the interactions between pathogens, commensals, and immunity operate at every level, from genes to tissues and systemic cell populations. Learning more about each of the players and their molecular and cellular interactions will be essential if we are to avoid any unintended consequences of antihelminth drug treatment or of live helminth therapy in humans (*12*). ■

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