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A new therapeutic strategy for malaria: targeting T cell exhaustion

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Abstract

Boosting immune responses during malaria remains a challenge. Overcoming T cell exhaustion by blocking coinhibitory receptors offers a promising lead.

Malaria is an increasing global problem, and resistance to the present antimalarial drugs has necessitated new therapeutic approaches¹. Understanding host responses to the *Plasmodium* species that cause malaria is critical for the rational design of effective antimalarial therapies and vaccines. The malaria parasite has evolved many mechanisms with which to evade immune responses, including a period of obligate intracellular growth in immune-privileged liver cells, plasmodial antigen switching among the 60 variations of the *Plasmodium falciparum* erythrocyte membrane protein PfEMP1, and inhibition of immune responses. The erythrocytic (blood) stage of malaria accounts for most of the immunopathology and mortality of malaria. Human and rodent studies have shown that CD4⁺ T cells and antibodies are important for protective immunity to blood-stage malaria¹. In this issue of *Nature Immunology*, Butler *et al.* examine immune responses during malaria infection and find that *Plasmodium*-specific T cells show features of T cell exhaustion².

Exhausted T cells develop in the setting of persistent antigen exposure, which drives a program of gene expression distinct from that of naive, memory or activated T cells and causes pathogen-specific T cells to lose functional activity^{3,4}. First described in CD8⁺ T cells in the mouse model of chronic infection with lymphocytic choriomeningitis virus, T cell exhaustion is now recognized as a general characteristic of chronic viral infections, including infection with human immunodeficiency virus, hepatitis C virus or hepatitis B virus. Exhausted T cells can express many coinhibitory receptors⁵ (Fig. 1). PD-1 (CD279) is the best-characterized coinhibitory receptor expressed during chronic infection; it is a mediator of immune dysfunction and disease progression^{6,7}. PD-1 has two ligands, PD-L1 (B7-H1 or CD274) and PD-L2 (B7-DC or CD273). PD-L1 expression is upregulated by interferons and is broadly induced on hematopoietic and nonhematopoietic cells. Signaling through PD-1 attenuates T cell antigen receptor signals and inhibits the cytokine production and cytolytic function of T cells. Blockade of PD-1 or PD-L1 during chronic viral infection can restore T cell function and diminish the viral load. The identification of exhausted T

cells in malaria provides novel mechanistic insights and suggests a new therapeutic approach for malaria.

Using *Plasmodium yoelii*, which causes a relatively acute infection that can be detected in the blood for about 5 weeks but is eventually cleared, Butler *et al.* study *Plasmodium*-specific antibody and T cell responses². Butler *et al.* show that CD4⁺ T cells and antibody responses are critical for a successful antimalaria immune response, but CD8⁺ T cells are not. Because tetramers of major histocompatibility complex (MHC) are not available for plasmodial antigens, the authors confirm the utility of surrogate markers (CD49^{hi}CD11a^{hi} T cells) and use these to examine activated *Plasmodium*-specific T cells. *Plasmodium*-specific T cells show functional T cell exhaustion with less production of cytokines in response to mitogens. Although exhaustion was initially described in CD8⁺ T cells, subsequent work has shown that CD4⁺ T cells also can become exhausted. Exhausted *Plasmodium*-specific CD4⁺ T cells have high expression of the coinhibitory receptors PD-1 and LAG-3 (CD223)⁸ but not 2B4 or CD160 (Fig. 1). LAG-3 binds to MHC class II proteins with a higher affinity than does CD4. Expressed on activated T cells, regulatory T cells, plasmacytoid dendritic cells and some natural killer cells, LAG-3 negatively regulates T cell activation and proliferation. In contrast, exhausted *Plasmodium*-specific CD8⁺ T cells have high expression of PD-1, LAG-3, 2B4 and CD160. A report examining human immunodeficiency virus-specific exhausted T cells has found similar differences between exhausted CD4⁺ and CD8⁺ T cells in coinhibitory receptor expression⁹.

As exhausted *Plasmodium*-specific CD4⁺ T cells have abundant expression of PD-1 and LAG-3, and CD4⁺ T cells are important for an effective anti-*Plasmodium* response, Butler *et al.* examine whether blockade of LAG-3 and PD-L1 (the main ligand for PD-1), alone or together, enhances antimalarial immunity². They quantify *P. yoelii* parasites in blood and find that combined blockade of PD-L1 and LAG-3 leads to an immediate halt to the increase in blood parasites and accelerates parasite clearance. Blockade of PD-L1 alone is moderately effective, but blockade of LAG-3 alone has little effect. Dual blockade leads to more *Plasmodium*-specific CD4⁺ T cells and CD8⁺ T cells, which produce more cytokines and enhance parasite control during blood-stage infection in both inbred and outbred mice. Dual blockade also leads to much more protective antibody, as demonstrated by experiments showing that transfer of serum from treated mice accelerates parasite clearance in naive recipients. *Plasmodium*-specific MSP1 immunoglobulin G titers are 2.5-fold higher. Consistent with that enhanced antibody response, the number of follicular helper T cells is increased by sevenfold, and that of plasmablasts, by 50-fold. Why might there be synergy between blockade of PD-L1 and blockade of LAG-3? One possibility is that blockade of PD-L1 upregulates IFN- γ production and the greater abundance of IFN- γ may lead to higher expression of MHC class II, a ligand for LAG-3. Therefore, blockade of PD-L1 may increase LAG-3 inhibitory signaling, creating a situation in which dual blockade would act synergistically.

Butler *et al.* also examine infection of mice with *Plasmodium chaubadi*, because this models chronic recrudescence malaria in humans, which can last for years². *P. chaubadi* establishes a persistent infection. Parasites are below the limit of detection by blood smear after 3 weeks, but persist, as blood will transfer infection. Combined blockade of PD-L1 and LAG-3 results in sterilizing immunity in which blood does not transmit infection, which suggests that dual blockade can successfully eliminate a low, persistent infection.

The findings noted above are relevant to human malaria. Butler *et al.* examine PD-1 expression on CD4⁺ T cells in children in Mali in the dry season before malaria and in the rainy period 1 week after diagnosis and treatment of the first *P. falciparum* infection of the malaria season². They find more PD-1 expression, from about 1% of CD4⁺ T cells to 3%,

which suggests an exhausted immune response in human malaria and the therapeutic potential of blockade of the coinhibitory pathway.

This paper adds malaria to the growing list of chronic infections in which T cell coinhibitory receptors limit pathogen eradication². One of the surprises in immunology of the past 15 years has been the large number of T cell-inhibitory pathways that come into play after immune activation. The initiation of an immune response has a high activation threshold, with the innate immune system surveying the environment and upregulating costimulatory molecules when infection is detected. Once initiated, the immune response is immensely powerful, with the ability to purge infection but also with the potential to cause great tissue damage. Thus, the extent of immune activation needs to be highly regulated to control immune-mediated tissue damage. Inhibitory pathways serve to terminate an immune response and resolve inflammation.

The same coinhibitory receptors involved in T cell exhaustion during chronic microbial infections also participate in tumor-specific T cell exhaustion^{10,11}. Incipient cancers are surveyed and can be eliminated by the immune system. When a tumor evades eradication by the immune response, persistent expression of tumor antigens can drive development of tumor-specific T cell exhaustion. The first coinhibitory receptor shown to restrain anti-tumor immunity was CTLA-4. The exciting result has been that blockade of CTLA-4 allows reactivation of T cell responses and more-effective immune response. Ipilimumab, a blocking CTLA-4-specific antibody, has been shown to improve the survival of patients with melanoma and is now a cancer immunotherapy approved by the US Food and Drug Administration¹². Clinical trials of PD-1 are in progress and have shown promise for a range of tumors¹³. Cancer immunotherapy with antagonists of other coinhibitory pathways is an active area of investigation. Blocking antibodies provide proof of principle and a first generation of therapeutics, but intensive effort is needed to identify small-molecule antagonists that are affordable and easily delivered.

Because exhausted T cells often express multiple coinhibitory receptors, a critical goal of the next few years will be to examine the effect of blocking combinations of coinhibitory pathways and to determine the efficacy of such therapy, alone and in combination, in chronic infection and cancer. Dual blockade of PD-L1 and LAG-3 or of PD-L1 and TIM-3 is synergistic in both chronic infection and cancer^{10,11,14}. Further work is needed to establish which additional combinations are synergistic, additive or antagonistic. Because many coinhibitory pathways also have a role in peripheral tolerance, blockade of coinhibitory pathways has the potential to break tolerance and elicit self-reactive immunopathology. It will be important to determine which combinations have an acceptably small amount of adverse effects.

The development of antibiotic resistance has shown that targeting a single mechanism of action allows more rapid development of resistance than does targeting two independent mechanisms; consequently, targeting a coinhibitory pathway and a second, distinct pathway may be more effective. Multiple inhibitory mechanisms regulate exhausted T cells. Studies have illustrated the therapeutic potential of combining blockade of a coinhibitory pathway with a complementary approach to boost immune responses. One effective approach is targeting a coinhibitory receptor and an inhibitory cytokine; dual blockade of the PD-1 and interleukin 10 (IL-10) pathways acts in synergy to promote viral clearance in the model of chronic infection with lymphocytic choriomeningitis virus¹⁵. A second promising approach is to combine immunostimulators with blockade of a coinhibitory pathway. The efficacy of therapeutic vaccination, which aims to enhance antimicrobial immune responses during chronic infection, has been disappointing; however, combined blockade of PD-L1 and therapeutic vaccination synergistically enhance T cell immunity and viral clearance.

Additional immunostimulatory approaches would include vaccine adjuvants such as ligands for Toll-like receptors or agonistic agents such as monoclonal antibody to the T cell–activation molecule 4-1BB. Combination therapies also may allow use of doses of some agents that are tolerated better by patients. For example, administration of IL-2 in conjunction with blockade of PD-L1 might permit the use of lower, nontoxic amounts of IL-2. Perhaps most promising may be to combine blockade of a coinhibitory receptor with targeted drug therapy, such as inhibitors of the serine-threonine kinase B-Raf in cancer or new-generation antimicrobial drugs in chronic infection.

In conclusion, T cell exhaustion is proving to be a common mechanism of immunoevasion in chronic infection and cancer. The identification of T cell exhaustion in malaria offers a much needed and novel therapeutic strategy for this devastating disease. The approval of CTLA-4 blockade for melanoma is a turning point in immunotherapy. The blockade of coinhibitory pathways alone or in combination offers great hope for the treatment of chronic infections and cancer.

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Figure 1.

T cell exhaustion. **(a)** Exhausted $CD4^+$ T cells in malaria upregulate PD-1 and LAG-3. Combined blockade of PD-L1 and LAG-3 allows more $CD4^+$ T cell activation and the provision of help by $CD4^+$ T cells for B cell antibody production. LAG-3 is a transmembrane protein composed of four immunoglobulin domains with a structure similar to that of the coreceptor CD4 but a higher affinity for MHC class II. The first immunoglobulin domain contains an extra loop region important for binding to MHC class II. The cytoplasmic domain contains a KIEELE motif that is required for LAG-3-mediated immunoinhibition. PD-1 contains a single immunoglobulin V domain and two tyrosine-phosphorylation motifs in its cytoplasmic domain that engage the tyrosine phosphatase SHP-2. ITSM, immunoreceptor tyrosine-based switch motif; ITIM, immunoreceptor tyrosine-based inhibitory motif; CD3, signaling complex of the TCR; APC, antigen-presenting cell. **(b)** Coinhibitory pathways. Exhausted T cells can express multiple inhibitory receptors, which are called 'coinhibitory' because they modify the outcome of a T cell antigen receptor signal and limit the population expansion, functional activity and survival of T cells. $CD4^+$ T cells and $CD8^+$ T cells express overlapping but distinct coinhibitory receptors. During malaria infection, CD244 and CD160 are expressed on exhausted $CD8^+$ T cells but not on exhausted $CD4^+$ T cells. PtdSer, phosphatidylserine.