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CD28 Costimulation Is Required for the Expression of T-Cell-Dependent Cell-Mediated Immunity against Blood-Stage Plasmodium chabaudi Malaria Parasites

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Mice suppress the parasitemia of acute blood-stage Plasmodium chabaudi malaria by an antibody- or T-cell-dependent cell-mediated mechanism of immunity (AMI and CMI, respectively) or by both mechanisms. To determine whether CD28 costimulation is required for expression of these polar immune responses, we first compared the time courses of P. chabaudi malaria in CD28-deficient (CD28−/−) and CD28-intact (CD28+/+) mice. Acute infections in both knockout (KO) and control mice followed similar time courses, with the period of descending parasitemia being prolonged ~2 weeks in KO mice followed by intermittent low-grade chronic parasitemia. Infected CD28−/− mice produced primarily the immunoglobulin M antibody, which upon passive transfer provided partial protection against P. chabaudi challenge, suggesting that the elimination of blood-stage parasites by CD28−/− mice was achieved by AMI. To determine whether CD28−/− costimulation is required for the expression of CMI against the parasite, we compared the time courses of parasitemia in B-cell-deficient double-KO (JH−/− × CD28−/−) mice and control (JH−/− × CD28+/+) mice. Whereas control mice suppressed parasitemia to subpatent levels within ~2 weeks postinoculation, double-KO mice developed high levels of parasitemia of long-lasting duration. Although not required for the suppression of acute P. chabaudi parasitemia by AMI, CD28 costimulation is essential for the elimination of blood-stage parasites by CMI.

An immunization-based approach to control malaria continues to be most appealing in light of cost-effectiveness, capacity for widespread implementation, and potential for sustained protection from disease. However, the ability to protect human subjects against Plasmodium falciparum by immunization or immunotherapy may require an understanding of antimalarial host immune responses beyond what is currently known (19, 32). Protective immune responses to malaria involve the participation of both innate and adaptive immune systems, in which a variety of cell types, including macrophages, dendritic cells, NK cells, B and T lymphocytes, and the molecules they secrete and/or display have crucial functions (reviewed in reference 28). Based on findings obtained from studies of malaria in human and animal hosts, blood-stage malaria parasites can be eliminated by antibody- and cell-mediated mechanisms of immunity (AMI and CMI, respectively) (reviewed in references 18, 25, and 42).

Efforts to understand immunity to malaria have focused primarily on the identity and function of protective antibodies in human subjects and animal hosts (28). Although antibody-independent T-cell-dependent immunity against blood-stage malarial parasites was first described for B-cell-deficient chickens more than 20 years ago (10, 31) and, subsequently, for mice made B cell deficient by lifelong treatment with anti-immunoglobulin M (IgM) (13, 39) or gene knockout (KO) (44, 49), the role of T-cell-dependent CMI in human malaria has been largely overlooked. Recently, however, Pombo et al. (29) reported that human subjects immunized with low doses of live P. falciparum blood-stage parasites were protected against challenge with viable blood-stage parasites. The protected subjects did not produce detectable antibodies in response to immunization, leading the authors to suggest that their subjects were protected by as yet undefined mechanisms of CMI against the parasite.

During experimental P. chabaudi malaria, protective antigens are presented to CD4+ T cells by major histocompatibility complex II glycoproteins expressed on the surfaces of antigen-presenting cells (7). Based on what is presently known about T-cell activation (reviewed in reference 26) a second signal provided by one or more of several costimulatory molecules leads to the activation of the responding CD4+ T cell and the subsequent development of parasite-eliminating effector mechanisms.

CD28 is an important and well-characterized costimulatory molecule that, following interaction with the B7 family of molecules, promotes the differentiation and activation of both naive CD4+ and CD8+ T cells and the generation and/or maintenance of memory cell populations. CD28 costimulation reduces the number of T-cell receptors (TCRs) that must be
triggered to achieve T-cell activation, and enhances T-cell survival and proliferation, as well as the production of cytokines and cytokine receptors. Also, it plays important roles in the generation of helper-T-cell subsets, germlinal center formation, antibody production, isotype switching, and somatic hypermutation (reviewed in references 16 and 37).

The significance of CD28 costimulation in the development of immunity to different infectious diseases is dependent on the infecting agent. It plays an essential role in acute infections caused by Salmonella enterica serovar Typhimurium (22), Listeria monocytogenes (23), Trypanosoma cruzi (24), and chronic Toxoplasma gondii infections (47) but has little, if any, role in the development of immunity to acute Toxoplasma gondii infection (47) and infections caused by Leishmania major (4) and lymphocytic choriomeningitis virus (36). Although the requirement for CD28 costimulation for the development of immunity to malaria has not been examined directly, Taylor-Robinson and Smith (41) reported that the treatment of P. chabaudi-infected mice with a CD86-blocking monoclonal antibody (MAb) prevented the curing of acute parasitemia observed in control mice, suggesting a possible role for CD86/CD28 costimulation in the control of chronic malaria.

Because the polyclonal responses of AMI and CMI against blood-stage malaria parasites may both represent viable targets for immunization, we have now analyzed the contribution of CD28 costimulation to the expression of AMI and CMI against acute P. chabaudi malaria. The data indicate that AMI is partially dependent on CD28 costimulation whereas CD28 costimulation is crucial for the expression of CMI against the parasite.

MATERIALS AND METHODS

Mice. Female wild-type (WT) C57BL/6 mice, TCR δ chain KO (δ−/−) mice, and CD28 KO (CD28−/−) mice were purchased from the Jackson Laboratories, Bar Harbor, Maine. CD28−/− and δ−/− mice had been backcrossed to C57BL/6 mice for eight and four generations, respectively. The original breeding pairs of B-cell-deficient J Ab−/− mice on a C57BL/6;129 background were kindly provided by Dr. H. Usuki (GenPharm International, Mountainview, Calif.). Progeny of these mice were maintained and bred at the Peter B. Medawar Laboratory for Medical Research (Madison, Wis.). The generation of double-KO J Ab−/− × CD28−/− and J Ab−/− × δ−/− mice and the identification of B-cell deficiency were achieved as described previously (50). The genotyping of mutated and WT CD28 genes was accomplished by standard PCR techniques using a modification of the protocol supplied by the Jackson Laboratories.

Parasites and infections. The nonlethal parasite strain P. chabaudi asaudi 556KA, hereafter referred to as P. chabaudi, was maintained as frozen stablate material and used as described previously (6). Unless noted otherwise, infections were initiated in mice by the intraperitoneal (i.p.) injection of 107 parasitized erythrocytes. Mice receiving antibody treatment by i.p. injection were anesthetized with methoxyflurane (Metofane) and injected intravenously (i.v.) with 107 parasitized erythrocytes. In most instances, parasitemia was assessed, beginning on day 5 postinoculation, by enumerating 200 to 1,000 erythrocytes on Giemsa-stained films of tail blood obtained every other day during the course of infection. The percent parasitemia was calculated from the equation percent parasitemia = (number of parasitized erythrocytes/total number of erythrocytes counted) × 100.

Cytokine detection. Splenic extracts for cytokine determinations were prepared from the spleens of CD28−/− and C57BL/6 mice on days 0, 3, 10, and 18 after inoculation with 107 P. chabaudi-parasitized erythrocytes. Control double-KO mice were injected with control sera from uninfected CD28−/− mice and challenged identically. Beginning day 3 postinoculation, the time course of parasitemia was monitored as described above.

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RESULTS

Experimental malaria in CD28 KO mice. To ascertain infection outcomes in mice deficient in CD28 costimulation, we infected CD28−/− mice and CD28 intact control mice with P. chabaudi-parasitized erythrocytes as described above. The time courses of acute parasitemia in the two groups of mice were similar, with peak parasitemia of approximately 9 to 11%, occurring between days 7 and 9 (Fig. 1). Whereas CD28-intact mice suppressed parasitemia to subpatent levels by day 13 postinoculation, CD28−/− mice suppressed parasitemia more slowly than CD28−/− mice and developed chronic low-grade parasitemia ranging between 0 and 0.1% throughout the 27-day period of observation. Nearly identical results were obtained in two replicate experiments involving a total of seven additional CD28−/− mice.

Cyttofluorimetric analysis of type 1 double-KO mice. To determine whether the absence of CD28 signaling would influence the expansion of lymphocyte subsets in the spleens of P. chabaudi-infected mice, spleen cells from infected and uninfected KO and control mice harvested between days 11 and 13 postinoculation were subjected to cytofluorimetric analysis. The results (Fig. 2) reveal that comparable expansions of all cell types examined, including CD4+ T cells, CD8+ T cells, γδ T cells, B cells, and NK cells, occurred in CD28−/− and control mice in response to infection. During the course of P. chabaudi infection, levels of expression of the activation marker B220 on γδ T cells (8) in CD28−/− and CD28-intact mice were similar (data not tabulated).

IFN-γ and TNF-α production by CD28−/− mice infected with P. chabaudi. To determine the effect of CD28 KO on the production of proinflammatory cytokines in response to infection, we measured IFN-γ and TNF-α concentrations in splenic extracts obtained during the course of infection in CD28−/− and WT mice. The results (Fig. 3) indicate that peak concentrations of both cytokines were present on day 10 postinoculation, with CD28−/− mice making approximately one-third as much IFN-γ as WT mice. In contrast, similar concentrations of TNF-α (~80%) were produced by CD28−/− mice and WT mice.

Antibody response of P. chabaudi-infected CD28−/− mice. To determine whether CD28−/− mice produced antibodies specific for parasite antigens in response to infection with P. chabaudi, sera were harvested from infected CD28−/− and control mice (n = 3) prior to inoculation with 10⁶ parasitized erythrocytes and at 42 days postinoculation. The sera were subsequently assayed by ELISA for isotype-specific antibodies reactive with P. chabaudi recombinant AMA-1 and MSP-1 42 antigens. The results (Table 1) indicate that CD28−/− and
control mice made antibodies against both AMA-1 and MSP-142 in response to infection, with more antibodies being produced against MSP-142 than against AMA-1 by both KO and control mice. The total antibody response of the CD28 KO control mice against AMA-1 and MSP-142 was approximately three- to fourfold greater, respectively, than that seen in CD28−/− mice. Whereas control mice produced antibodies representing all of the isotypes assayed, with IgG2b and IgG2c dominating, IgM accounted for >90% of the antibodies measured in the sera of infected CD28−/− mice.

**Passive immunization against P. chabaudi infection with sera obtained from infection-immunized CD28−/− mice.** To determine whether the sera from infection-immunized CD28−/− mice were capable of protecting recipient mice against challenge with viable *P. chabaudi* parasites, double-KO (*Jh−/− × δ−/−*) mice deficient in B and γδ T cells were passively immunized with immune or nonimmune sera pooled from infection-immunized CD28−/− or uninfected CD28−/− mice, respectively, and challenged with viable *P. chabaudi* parasites as described above. The results (Fig. 4) indicate that the time course of ascending parasitemia in recipients given immune sera was delayed by ~3 days in comparison to that in recipients given nonimmune sera. Mean parasitemia values for four recipients of immune sera with patent parasitemia were significantly different (*P* < 0.05), from those for recipients of nonimmune sera on days 9, 11, and 13 during ascending parasitemia, reflecting the decrease in inoculum size, presumably due to antibodies in the immune sera. With the exception of one recipient of immune sera that failed to develop patent parasitemia until 28 days postinoculation, both groups of recipient mice developed peak parasitemia on day 15 postinoculation. We do not know why the appearance of patent parasitemia was delayed in one mouse. Thereafter, parasitemia remained elevated in all recipient mice throughout the 30-day observation period. A similar delay in the course of ascending parasitemia in recipients of immune sera obtained from CD28−/− mice was observed in a replicate experiment. The course of ascending parasitemia in *Jh−/− × δ−/−* mice that had received sera from infection-immunized CD28−/− mice paralleled that in the recipients of nonimmune sera but was delayed by ~3 days (data not tabulated).

**Time course of *P. chabaudi* parasitemia in *Jh−/− × CD28−/− mice.** To determine whether CMI against the parasite remained intact in the absence of CD28 signaling, we infected *Jh−/− × CD28−/−* mice and *Jh−/− × CD28+/−* control mice i.p. with 10⁶ *P. chabaudi*-parasitized erythrocytes. The slopes of ascending parasitemia and the magnitudes of peak parasitemia in both KO and control mice were nearly identical. Whereas control mice suppressed their acute infections as described previously (44), double-KO mice, deficient in both B cells and functional CD28 genes, were unable to do so; instead, they developed levels of parasitemia that remained elevated throughout the 32-day observation period (Fig. 5). Identical results were obtained with CD28−/− mice made B cell deficient by lifelong treatment with goat anti-mouse IgM (data not tabulated).

**DISCUSSION**

We have now dissected the role of CD28 costimulation in the activation of AMI and CMI against *P. chabaudi* blood-stage malaria. CD28−/− mice, in comparison to CD28-intact control mice, were slow to suppress *P. chabaudi* parasitemia despite making measurable antibodies against parasite antigens. When CD28−/− mice were made B cell deficient by gene KO, they developed high levels of unremitting parasitemia, which failed to cure. Taken together these observations indicate that CD28 costimulation contributes to the expression of AMI against blood-stage malaria parasites and is absolutely essential for CMI against *P. chabaudi*.

Taylor-Robinson and Smith reported that blockade of CD80

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**TABLE 1. Isotypic profile of *P. chabaudi* AMA-1 and MSP-142 antibodies induced by *P. chabaudi* infection of C57BL/6 and CD28−/− mice**

<table>
<thead>
<tr>
<th>Protein and mouse type</th>
<th>IgM</th>
<th>IgG1</th>
<th>IgG2b</th>
<th>IgG2c</th>
<th>IgG3</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMA-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C57BL/6</td>
<td>508 ± 438</td>
<td>86 ± 4</td>
<td>676 ± 557</td>
<td>1,417 ± 410</td>
<td>235 ± 127</td>
</tr>
<tr>
<td>CD28 KO</td>
<td>1,013 ± 770</td>
<td>&lt;2</td>
<td>48 ± 28</td>
<td>102 ± 56</td>
<td>&lt;2</td>
</tr>
<tr>
<td>MSP-142</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C57BL/6</td>
<td>1,377 ± 961</td>
<td>140 ± 101</td>
<td>1,492 ± 1,286</td>
<td>2,730 ± 2,141</td>
<td>1,948 ± 1,037</td>
</tr>
<tr>
<td>CD28 KO</td>
<td>4,961 ± 3,991</td>
<td>22 ± 8</td>
<td>39 ± 23</td>
<td>130 ± 112</td>
<td>708 ± 81</td>
</tr>
</tbody>
</table>

**FIG. 4. Time course of *P. chabaudi* infection in individual *Jh−/− × δ−/−* mice passively immunized with serum obtained from infection-immunized CD28−/− mice (solid lines) or serum from uninfected CD28−/− mice (broken lines).** Mice were injected with immune or normal serum in a dose of 0.5 ml i.p. on days −1, 0, and +1 relative to inoculation i.v. with 10⁶ *P. chabaudi*-parasitized erythrocytes. Different symbols represent data for different mice.
or CD86 with specific MAbs had little if any effect on the time course of acute *P. chabaudi* parasitemia (41). However, mice treated with anti-CD86 developed chronic malaria, characterized by low-level parasitemia of prolonged duration. Blockade of CD80, in contrast, was without effect on the time course of infection, allowing these authors to conclude that CD28 costimulation did not play an essential role in the suppression of acute parasitemia. Rather, the activation of T cells responsible for this function was achieved via other costimulatory pathways. They proposed, however, that the interaction of CD86 with CD28 was necessary for the total elimination of blood-stage parasites, thereby preventing chronic malaria. Our results support this contention; CD28 KO mice infected with the less-virulent *P. chabaudi adami* utilized in our study suppressed acute infections in the same time frame as control mice but subsequently developed chronic parasitemia, which remained patent for several weeks after CD28-intact control mice had eliminated blood-stage parasites. Although Green et al. (12) were unable to find evidence for B-7-independent responses in the absence of CD28, Mandelbrot and colleagues (20, 51) have reported CD28-independent but B7-dependent costimulation of T cells in response to cardiac allografts. Our observation that chronic malaria developed in CD28−/− mice, together with the observations of Taylor-Robinson and Smith (41), indicates that the absence of CD28, and not another costimulatory molecule capable of signaling through B7, is indeed responsible for the emergence of chronic parasitemia.

Although CD28 signaling has been reported to be essential for the clonal expansion and survival of CD4+ naïve T cells (37), *P. chabaudi* infection induced nearly identical changes in the expansion of like splenic lymphocyte populations in both CD28−/− mice and WT controls. These observations indicate that the changes seen in these lymphocyte populations occurred in the absence of CD28 costimulation. The recently described ICOS/B7 costimulation system (14, 52) in addition to the costimulating molecules considered by Taylor-Robinson and Smith (41) may account for CD28-independent costimulation. The CD28/B7 and ICOS/B7 signaling pathways are thought to parallel one another and appear to have a redundant function in CD4+ T-cell activation mediating resistance to *Toxoplasma gondii* (48). It is also possible that high doses and continuous delivery of parasite antigens during *P. chabaudi* parasitemia may compensate for the lack of CD28 costimulation, if indeed CD28 costimulation provides quantitative support for TCR signaling, as proposed by Acuto and Michel (1).

As indicated above, the elimination of blood-stage *P. chabaudi* parasites can be achieved by AMI and/or CMI (13, 39, 40, 43, 44, 49, 50). Previous studies have reported that B-cell-deficient mice suppressed acute parasitemia to sub-patent levels in the same time frame as B-cell-intact mice but subsequently developed low-grade chronic parasitemia, having a prolonged duration ~2 years (W. P. Weidanz, unpublished data). B-cell-intact mice displayed one or more waves of parasitemia before sterilizing their infections. When CD4 αβ T cells or γδ T cells in JH−/− mice infected with *P. chabaudi* were depleted by T-cell-specific MAb treatment or gene knockout, the mice did not suppress acute parasitemia (35, 41, 50). These findings indicate that both cell types are required for the expression of CMI against the parasite, with γδ T-cell activation being dependent on CD4+ cells responding to malarial antigens (45). The observations that both CD28−/− mice and anti-B7-blockaded mice suppressed the parasitemia of acute malaria in the same time frame as WT control mice did not clarify whether this event was accomplished via AMI or CMI or both in the absence of CD28 costimulation. Parasite-specific antibodies against both AMA-1 and MSP-1 42 were present in the sera of both infected CD28−/− and WT mice but differed both quantitatively and qualitatively depending on the type of mouse making the response, the specific test antigen used to measure the antibody, and the isotype of the antibody being measured. The antibody response made by CD28−/− mice to both antigens was significantly less than that observed in WT controls and consisted primarily of IgM, in contrast to the presence of antibodies of different isotypes in the sera of WT mice, suggesting that Ig isotype switching was deficient in the KO mice.

Passive immunization of JH−/− × δ−/− recipients with sera from infection-immunized CD28−/− mice provided partial protection against challenge, as indicated by a delay in the onset of patent parasitemia in the recipients of immune versus nonimmune sera. Based on our serological findings, these data suggest that a component of immune sera, presumably the IgM antibody, mediated the observed protection. Thus, AMI against *P. chabaudi* may be isotype independent. Previously, we were unable to correlate the concentrations of antibody isotypes in the sera of mice immunized with antigenic constructs to protection against *P. chabaudi* challenge (5). Having reported that the passive transfer of Ig-rich fractions of immune sera harvested from infection-immunized δ−/− mice protected JH−/− × δ−/− recipients against *P. chabaudi* challenge (50), we believe that it is unlikely that cytokines/chemokines in the immune sera may have activated CD4+ T cells in the JH−/− × δ−/− recipients, resulting in the observed protection. In addition, our previous findings indicate that *P. chabaudi* parasitemia was not suppressed in B-cell-deficient mice depleted of γδ T cells despite the expansion of the CD4+ T-cell population in response to infection (45). By comparing the time courses of *P. chabaudi* malaria in JH−/− × CD28−/− and JH−/− × CD28-/+ control mice, we were able to examine the effects of CD28 costimulation on the development of CMI against the
parasite in the absence of confounding antibodies. Unlike the self-curing acute malaria in CD28\(^{-/-}\) mice, unremitting high levels of parasitemia for prolonged duration were observed in double-KO mice, indicating an essential role for CD28 co-stimulation for the expression of CMI against the parasite. The absence of antiparasite CMI in P. chabaudi-infected CD28\(^{-/-}\) mice suggests a differential requirement for CD28 co-stimulation in the development of immunity against blood-stage malaria parasites, wherein AMI impaired by a deficiency in IgG isotype antibodies is sufficient to suppress acute parasitemia but unable to prevent chronic parasitemia. The development of CMI against P. chabaudi, in contrast, is totally dependent on CD28 co-stimulation. Our observation that J\(H^+\) \(\times\) CD28\(^{-/-}\) mice in contrast to J\(H^+\) \(\times\) CD28\(^{+/+}\) B-cell-deficient controls and CD28\(^{-/-}\) mice failed to suppress acute parasitemia provides additional evidence suggesting that CD28\(^{-/-}\) mice cure acute P. chabaudi infections by means of AMI. We have also observed that the time course of nonlethal Plasmodium yoelii infections in CD28\(^{-/-}\) mice was extended approximately 7 days compared to that in CD28-intact controls (W. F. Weidanz, unpublished data). Because the normally avirulent P. yoelii infections are lethal in B-cell-deficient mice (44), we could not determine the effects of CD28 co-stimulation deficiency on the development of CMI against this parasite in the J\(H^+\) mouse.

These results pose an interesting question as to why CMI against blood-stage P. chabaudi is deficient in CD28\(^{-/-}\) mice while AMI against the parasite appears to be functioning to a measurable degree in these mice. Although the mechanisms by which CD28 signaling leads to the development of CMI capable of eliminating blood-stage malaria parasites remain to be determined, the observations that CD4\(^+\) and \(\gamma\delta\) T-cell populations in infected KO mice expanded to the same extent as that observed in CD28-intact control mice suggests several possibilities. CD28\(^{-/-}\) T cells may fail to produce factors in sufficient amounts necessary for the activation of downstream effector mechanisms required for killing malaria parasites. In contrast to the findings of Taylor-Robinson and Smith (41), who reported that IFN-\(\gamma\) production by spleen cells of CD86-blockaded mice was enhanced, we observed that CD28\(^{-/-}\) mice produced measurable but significantly less IFN-\(\gamma\) (\(P < 0.05\)) than WT mice in response to infection. Based on observations that mice deficient in both IFN-\(\gamma\) and B cells were unable to suppress acute P. chabaudi parasitemia (2, 38, 46), IFN-\(\gamma\) appears to play an essential role in CMI against the parasite. Whether the IFN-\(\gamma\) deficiency observed in our present studies contributed to the observed defect in CMI is not known. Increasing TNF-\(\alpha\) levels in splenic extracts from infected CD28\(^{-/-}\) and WT mice were nearly identical throughout infection and did not appear to influence the development of CMI. Although increased TNF-\(\alpha\) levels have been associated with the severity of malaria in human subjects and in mice (18, 25, 28), studies of TNF receptor \(\alpha\) mice indicate little, if any, role for TNF-\(\alpha\) in immunity to acute P. chabaudi infection (17, 33).

Although the expansion and activation of the \(\gamma\delta\) T-cell population in CD28 KO mice did not appear to be perturbed by infection, these cells, which are essential for the expression of CMI against blood-stage P. chabaudi parasites in CD28-intact mice (35, 43) failed to kill parasites in J\(H^+\) \(\times\) CD28\(^{-/-}\) mice. It remains possible that other crucial cell types, such as NK cells (27), endothelial cells (15, 30), and professional antigen-presenting cells (16, 37), that express B7 may not be fully activated and/or functional during blood-stage malaria in the absence of CD28/B7 interactions, thereby impairing CMI against the parasites. Girvin et al. (11), studying experimental encephalomyelitis, reported that T cells from CD28\(^{-/-}\) mice produced amounts of IFN-\(\gamma\) and TNF-\(\alpha\) in response to the encephalitogenic peptide PLP56-70 equivalent to those produced by T cells of CD28-intact mice. Disease severity, however, in CD28\(^{-/-}\) mice was reduced and peptide-specific delayed-type hypersensitivity responses were significantly decreased in comparison to those in CD28-intact mice, leading these authors to suggest that CD28 co-stimulation in vivo played a critical role in cell trafficking and the recruitment of inflammatory cells.

In view of our findings, the concept of manipulating co-stimulation pathways to achieve levels of AMI or CMI sufficient to either eliminate blood-stage malaria parasites or produce low-grade parasitemia, which appears to be capable of maintaining immunity against disease, may be worthy of consideration. Such an approach is being used clinically in the immunotherapy of autoimmune diseases, malignant disease, allograft rejection, and the treatment of allergic disorders (37). Exploiting co-stimulation pathways for the immunoprophylaxis of human malaria is an attractive possibility. Indeed, Boutlis et al. (3) have recently suggested that augmentation of the short-lived IgG responses to P. falciparum glycosylphosphatidylinositolos may be achieved with costimulatory molecules.

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