

Letter to the Editor

The human polysaccharide- and protein-specific immune response to *Streptococcus pneumoniae* is dependent on CD4⁺ T lymphocytes, CD14⁺ monocytes, and the CD40–CD40L interaction

To the Editor:

Streptococcus pneumoniae is a major cause of pneumonia, septicemia, and meningitis. It causes great morbidity and mortality throughout the world. In particular, young children, the elderly, and immunocompromised individuals are susceptible to pneumococcal infections. Host protection against pneumococcal infections is mediated by antibodies against pneumococcal surface proteins and capsular polysaccharides (caps-PSs).¹ Caps-PSs are a main determinant of virulence of *S pneumoniae*, and antibodies to caps-PSs are protective against infection with *S pneumoniae*. Some pneumococcal surface proteins, such as pneumococcal surface protein A (PspA), are also considered virulence factors.

Whereas proteins are T lymphocyte-dependent antigens, caps-PSs are considered T lymphocyte-independent type 2 (TI-2) antigens. Although T lymphocytes are not needed for induction of the antibody response to TI-2 antigens, they are involved in the regulation of this antibody response.² The role of T lymphocytes in the regulation of the antibody production to TI-2 antigens is poorly understood. Little is known regarding the mechanisms by which intact *S pneumoniae* elicits protein- and caps-PS-specific antibodies in human subjects. In the present study we immunized humanized mice with severe combined immunodeficiency (SCID) with heat-inactivated *S pneumoniae* as a model to study the human antibody response to *S pneumoniae*.

To evaluate the role of CD4⁺ T cells in the antibody response to pneumococcal caps-PSs and PspA, 6- to 8-week-old SCID mice were treated with TM β 1 (an anti-murine IL-2 receptor β -chain antibody used to deplete murine natural killer cells and to improve transplant survival³) and transplanted (in peritoneum) either with PBMCs (containing 4×10^6 B lymphocytes, $n = 6$) or with PBMCs depleted of CD4⁺ cells (by using microbeads; Miltenyi Biotec, Bergisch Gladbach, Germany; $n = 6$). After transplantation, the mice were immunized with heat-inactivated *S pneumoniae* serotype 3 (2×10^8 colony-forming units). Two weeks after immunization, antibodies were measured by means of ELISA (with preabsorption of the sera with caps-PS serotype 22F [5 mg/L] for anti-caps-PS).^{4,5} SCID/SCID mice reconstituted with PBMCs, but not with PBMCs depleted of CD4⁺ cells, produced high levels of IgM and IgG antibodies against caps-PSs and PspA (data not shown; $P < .03$, Mann-Whitney U test).

SCID/SCID mice were reconstituted with human PBMCs and immunized with *S pneumoniae* serotype 3 in the presence ($n = 6$) or absence of antagonistic anti-CD40 (ch5D12, 500 μ g; PanGenetics, Utrecht, The Netherlands; $n = 6$) to investigate whether the CD40–CD40L interaction is important for the human IgM and IgG anti-caps-PS antibody production *in vivo*. Anti-CD40 totally blocked the IgM and IgG anti-PspA antibody response, as well as the anti-caps-PS antibody response ($P < .03$). Human polyclonal control antibodies did not affect the antibody response (data not shown). Similarly, anti-CD40 blocked

the antibody response to *S pneumoniae* serotype 1 and serotype 14 and the antibody response (IgM and IgG) to dextran, another TI-2 antigen (data not shown; $P < .03$, Mann-Whitney U test).

Next we examined whether the stimulatory effect of CD4⁺ T lymphocytes on the human polysaccharide- and protein-specific antibody response to *S pneumoniae* was the result of a direct effect of T lymphocytes on B lymphocytes or of an indirect effect through interaction of T lymphocytes with CD14⁺ mononuclear cells. SCID/SCID mice were reconstituted with (1) purified B lymphocytes, (2) purified B lymphocytes in combination with purified CD4⁺ T lymphocytes, or (3) purified B lymphocytes in combination with purified CD4⁺ T lymphocytes and purified CD14⁺ cells. The results are shown in Fig 1 and indicate that transfer of B lymphocytes, CD4⁺ T lymphocytes, and CD14⁺ antigen-presenting cells was needed for SCID/SCID mice to be able to produce polysaccharide- and protein-specific antibodies. Transfer of purified B and T lymphocytes was not sufficient to induce anti-caps-PS and anti-protein antibody production. The presence of CD14⁺ monocytes was needed. The anti-caps-PS and anti-PspA antibody production by B lymphocytes, T lymphocytes, and CD14⁺ mononuclear cells could be totally blocked with anti-CD40 mAbs ($P < .0003$). Taken together, these data suggest that CD40L expressing CD4⁺ T lymphocytes stimulate the anti-caps-PS and anti-protein antibody production through interaction with CD40-expressing non-B antigen-presenting cells.

Patients with X-linked hyper-IgM (XHIM) syndrome have a mutation at the *CD40L* gene and therefore lack expression of functional CD40L. Patients with XHIM syndrome have mainly IgM-producing CD27⁺IgD⁺IgM⁺ B lymphocytes and lack IgG-producing CD27⁺IgD⁺IgM⁺ B lymphocytes.⁶ SCID/SCID mice reconstituted with CD19⁺ B lymphocytes originating from a healthy blood donor and with PBMCs depleted of CD19⁺ B lymphocytes from a patient with XHIM syndrome completely failed to produce anti-caps-PS and anti-PspA antibodies after immunization with *S pneumoniae*. By contrast, SCID/SCID mice reconstituted with CD19⁺ B lymphocytes from a patient with XHIM syndrome in combination with PBMCs depleted of CD19⁺ B lymphocytes from a healthy blood donor produced IgM anti-caps-PS and anti-PspA antibody levels comparable with those seen in the control condition after immunization with *S pneumoniae* (data not shown). Only very low levels of IgG anti-caps-PS and anti-PspA antibodies were formed (data not shown).

In a previous study we demonstrated that the human antibody response to soluble caps-PS (pneumococcal vaccine) was dependent on the CD40–CD40L interaction.³ In the present study we evaluated the regulation of the human antibody response to *S pneumoniae*. More specifically, we investigated the role of CD4⁺ T cells and the CD40–CD40L costimulatory interaction in the protein (PspA)- and caps-PS-specific antibody responses to *S pneumoniae*. Consistent with studies in mice,⁵ we found that the IgG polysaccharide-specific and protein-specific response to *S pneumoniae* was dependent on CD4⁺ T lymphocytes and the CD40–CD40L interaction. In contrast to a previous study in mice,⁵ the human IgM anti-polysaccharide response to *S pneumoniae* was dependent on CD4⁺ T cells, CD14⁺ mononuclear cells, and the CD40–CD40L interaction. The IgM protein- and caps-PS specific antibody response was blocked by anti-CD40, and adoptive transfer of CD4⁺ T cells obtained from a patient with XHIM

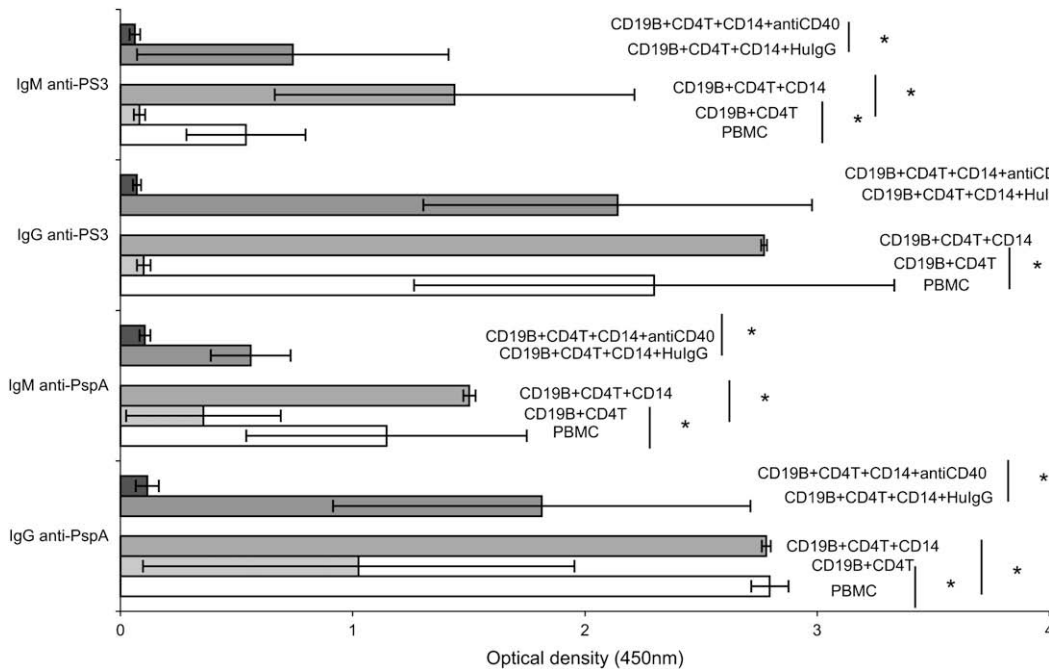


FIG 1. CD40L-expressing CD4⁺ T lymphocytes stimulate the human anti-caps-PS antibody response through interaction with CD40-expressing CD14⁺ mononuclear cells. SCID/SCID mice were reconstituted with (1) human PBMCs (n = 5); (2) purified CD19⁺ B lymphocytes and CD4⁺ T lymphocytes (n = 4); (3) purified CD19⁺ B lymphocytes, CD4⁺ T lymphocytes, and CD14⁺ monocytes in the presence of human polyclonal IgG (n = 4); or (4) anti-CD40 mAbs (n = 2). The purity of the cell populations was greater than 99% for all fractions. The mice were immunized with heat-inactivated *S pneumoniae* serotype 3. The absorbance values at serum dilution (1:100) are shown. The results shown are from 6 independent experiments. The error bars show the SD. *Statistically significant ($P < .05$, Mann-Whitney *U* test [Analyse-it for Microsoft Excel]).

syndrome failed to stimulate the IgM anti-protein and anti-caps-PS antibody response. Finally, we revealed that CD40L-expressing CD4⁺ T lymphocytes stimulate the human anti-caps-PS antibody response in an indirect way through interaction with CD40-expressing CD14⁺ monocytes. In murine studies on the antibody response to soluble caps-PS, a similar interaction between CD4⁺ T cells and antigen-presenting cells has been proposed.⁷ We hypothesize that the CD40-CD40L interaction between CD14⁺ mononuclear cells and CD4⁺ T cells could induce the release of soluble factors that can further stimulate B lymphocytes to produce IgM and IgG anti-caps-PS antibodies. A role for dendritic cells in the anti-caps-PS immune response has previously been suggested. Meltzer and Goldblatt⁸ showed that pneumococcal polysaccharides interact with human dendritic cells. Colino et al⁹ reported that bone marrow-derived dendritic cells pulsed with *S pneumoniae* could stimulate the anti-caps-PS antibody response when injected into naive mice and that endogenous T lymphocytes were critical for the development of IgG antibodies specific for both protein and polysaccharide antigens.

In conclusion, the data described in this report implicate a role for endogenous CD40-CD40L interactions in the human antibody response to TI-2 antigens in general and to intact heat-inactivated *S pneumoniae* in particular. CD40L-expressing CD4⁺ T lymphocytes stimulate the antibody response to *S pneumoniae* through interaction with CD14⁺ mononuclear cells.

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