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Essential Role for CD40 Ligand Interactions in T Lymphocyte-Mediated Modulation of the Murine Immune Response to Pneumococcal Capsular Polysaccharides

Axel Jeurissen,* Margreet Wyts,† Ahmad Kasran,‡ Sheema Ramdien-Murli,‡ Louis Boon,‡ Jan L. Ceuppens,† and Xavier Bossuyt‡*

Protection against infection with pneumococci is provided by anti-capsular polysaccharide (caps-PS) Abs. We investigated whether CD40 ligand (CD40L) plays a role in T lymphocyte-mediated regulation of the immune response to caps-PS, which are considered thymus-independent Ags. Administration of MR1, an antagonist mAb against murine CD40L, in BALB/c mice immunized with Pneumovax resulted in an inhibition of the IgM and IgG Ab response for various caps-PS serotypes. Evidence for the involvement of CD40+ T lymphocytes in the Ab response to caps-PS was obtained in SCID/SCID mice that, when reconstituted with B lymphocytes and CD4+ T lymphocytes, mounted a higher specific IgM response compared with SCID/SCID mice reconstituted with only B lymphocytes. This helper effect of CD4+ T lymphocytes was abrogated by MR1. Blocking CD40L in vitro decreased the IgM response to caps-PS and abolished the helper effect of CD4+ T lymphocytes. CD8+ T lymphocyte-depleted murine spleen cells mounted a higher in vivo immune response than total murine spleen cells, which provided evidence for a suppressive role of CD8+ T lymphocytes on the anti-caps-PS immune response. CD4+ T lymphocyte-depleted murine spleen cells, leaving a B and CD8+ T lymphocyte fraction, elicited only a weak in vivo and in vitro Ab response, which was enhanced after MR1 administration. In summary, our data provide evidence that T lymphocytes contribute to the regulation of the anti-caps-PS immune response in a CD40L-dependent manner. The Journal of Immunology, 2002, 168: 2773–2781.

Streptococcus pneumoniae is a microorganism that frequently causes pneumonia, septicemia, and meningitis (1). Espeically young children and the elderly are vulnerable for this pathogen with increasing antibiotic resistance (2).

Although pneumococcal cell wall polysaccharides are highly immunogenic, Abs to cell wall polysaccharides are not protective against invasive pneumococcal infection (3–5). Protection against S. pneumoniae is mainly provided by Abs to pneumococcal capsular polysaccharides (caps-PS) (6). Therefore, it has become of extreme importance to understand the mechanism by which protection to caps-PS is provided by the immune system.

The Ab response to pneumococcal polysaccharides is considered thymus independent (TI) because neonatally thymectomized mice and athymic nude mice are able to mount an Ab response similar in magnitude to that of conventional thymus-bearing mice (7–9). Because T lymphocytes or T lymphocyte products increase the Ab response to pneumococcal polysaccharides, pneumococcal caps-PS are classified as TI type 2 (TI-2) Ags (10). Whereas TI-2 Ags do not need T lymphocytes to induce an Ab response, thymus-dependent (TD) Ags (e.g., proteins) require participation of CD4+ T lymphocytes to generate an immune response (11). The immune response to TD Ags critically depends on the interaction between CD40 ligand (CD40L), a molecule which is transiently expressed on the surface of activated CD4+ T lymphocytes, and CD40 expressed on the surface of B lymphocytes (12). This interaction activates the resting B lymphocyte to produce Abs to TD Ags and is important for germinal center creation, memory B lymphocyte formation, and Ig class switching (13–17).

The question whether the CD40-CD40L interaction plays a role in the immune response to TI-2 Ags in general and pneumococcal polysaccharides in particular remains unsolved. There are contradictory data with regard to the role of CD40L in the immune response to TI-2 Ags after immunization with an intact extracellular microorganism or with a polysaccharide-protein conjugate. Wu et al. (18), using CD40L knockout mice, reported that the IgG response against phosphorylcholine (a TI-2 Ag) in mice immunized with a nonencapsulated variant of S. pneumoniae is T lymphocyte and CD40L dependent. However, it has also been described that a blocking anti-CD40L Ab (MR1) had little or only modest effect on the antiphosphorylcholine response and that CD40−/− dendritic cells elicited an anti-phosphorylcholine response that was comparable to that observed for wild-type dendritic cells (19). Szomolanyi-Tsuda et al. (20) stressed the role of the CD40-CD154 interaction in anti-polyoma virus thymus cell-independent IgG responses. Finally, it has been described 1) that neutralization of CD40L reduced the Ab response to pneumococcal caps-PS elicited by immunization with whole bacteria or with a caps-PS-protein conjugate and 2) that the immune response to a caps-PS-protein conjugate was higher in wild-type mice than in CD40L-deficient mice (21, 22). In contrast to immune responses to TI Ags on intact microorganisms or in polysaccharide-protein conjugates, immune

*Department of Molecular Cell Biology, Laboratory of Experimental Laboratory Medicine and "Department of Pathophysiology, Laboratory of Experimental Immunology, Faculty of Medicine, Catholic University Leuven, Leuven, Belgium; and †Department of Pathophysiology, Laboratory of Experimental Immunology, Faculty of Medicine, Catholic University Leuven, Leuven, Belgium.

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2 Address correspondence and reprint requests to Dr. Xavier Bossuyt, Laboratory Medicine, University Hospitals Leuven, GIBB-Heresstraat 49, B-3000 Leuven, Leuven, Belgium. E-mail address: Xavier.Bossuyt@uz.kuleuven.ac.be

3 Abbreviations used in this paper: caps-PS, capsular polysaccharide; TI, thymus independent; TD, thymus dependent; CD40L, CD40 ligand.

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responses to soluble polysaccharides are believed to be T lymphocyte and CD40L independent. Blocking the CD40-CD40L interaction with an anti-CD40L mAb did not inhibit the Ab formation against trinitrophenyl-conjugated Ficoll, a TI-2 Ag and CD40 and CD40L knockout mice mounted a normal Ab response to haptenated Ficoll (22–25). Recently, it was demonstrated that blocking CD40L in mice did not affect the Ab response against isolated pneumococcal caps-PS type 6B (21). However, there is indirect evidence that the CD40-CD40L interaction might be important for the immune response to soluble TI-2 Ags, since it was demonstrated that in vivo administration of trinitrophenyl-conjugated Ficoll induced a rapid expression of CD40L on CD4+ T lymphocytes (15). Furthermore, Abs that stimulate the CD40 molecule in mice augment the Ab response to purified pneumococcal caps-PS (26). It has been suggested that this stimulatory effect of this agonist anti-CD40 on the anti-caps-PS immune response is mainly due to increased activity of accessory cells that affect plasmablast growth and differentiation rather than mimicry of T lymphocyte help (27).

The present study was undertaken to determine the role of the CD40-CD40L interaction in the immune response to pneumococcal caps-PS Ags. We investigated whether the CD40-CD40L interaction between B cells and CD4+ and/or CD8+ T lymphocytes plays an important role in the regulation of the murine immune response to caps-PS.

Materials and Methods

Materials

Pneumovax, a 23-valent pneumococcal vaccine containing 25 μg of the caps-PS (Danish nomenclature) types 1–5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, and 33F, was obtained from Aventis Pasteur (Brussels, Belgium). Pneumococcal caps-PS and C polysaccharide were obtained from American Type Culture Collection (Manassas, VA). MR1, a monoclonal blocking Ab to murine CD40L, was provided by Macrozyme B.V. (Amsterdam, The Netherlands). Polyclonal hamster IgG was obtained from Biotrend Chemikalien (Köln, Germany). These Abs were purified using the Spectra/Por Float-A-Lyser from Spectrum Laboratories (Rancho Dominguez, Canada). Purified rat anti-mouse CD15 (TDS), PE anti-mouse CD4 (L3T4), PE anti-mouse CD8a (Ly-2), FITC polyclonal anti-mouse CD3e (CD3e chain), FITC polyclonal anti-rat Ig were purchased from BD Biosciences (Erembodegem-Aalst, Belgium). Peroxidase-conjugated goat anti-mouse IgM and IgG were obtained from Nordic Immunological Laboratories (Tilburg, The Netherlands). Goat serum and PBS were purchased from Life Technologies (Paisley, U.K.). 3,3′,5′-tetramethylbenzidine was purchased from DAKO (Heverlee, Belgium). HCl (1 N) and H2SO4 (3 N) solution were obtained from Merck (Darmstadt, Germany). Cell Strainers, 70-μm nylon, were supplied by BD Labware Europe (Le Pont De Claix, France). RPMI 1640 culture medium, glutamine, Tween 20 (polyoxyethylene sorbiton), agarose type I, HEPES, 50 μg/ml gentamicin, 5 × 10−7 M 2-ME, and 25% (v/v) T cell-replacing factor. T cell-replacing factor is defined as the supernatant of a 24-h culture of irradiated mouse T cells in the presence of PWM (29). HEPES, 50 μg/ml gentamicin, and 5 × 10−7 M 2-ME were added to the wells and incubated for 2 h at 37°C. After washing three times with 0.05% CFA of PBS, the plate was incubated for 1 h at 37°C. Thereafter, 3,3′,5′-tetramethylbenzidine was added for color development. After 15 min, the reaction was stopped by an acid stock solution that contained 1 N HCl and 3 N H2SO4. Plates were read at 450 nm.

In vitro cell culture

Mice were sacrificed under ether anesthesia and the spleen was removed. Mouse mononuclear cells were isolated from the spleen using a Cell Strainer. The lymphocytes were cultured in Falcon tissue culture flasks in RPMI 1640 supplemented with 10% FCS, 2 mM glutamine, 15 mM HEPES, 50 μg/ml gentamicin, 5 × 10−7 M 2-ME, and 25% (v/v) T cell-replacing factor. T cell-replacing factor is defined as the supernatant of a 24-h culture of irradiated mouse T cells in the presence of PWM (29). PWM was removed by incubating 1 × 106 erythrocytes per PWM for 1 h at 37°C (30). Cells were cultured at a density of 1 × 106 per ml and were stimulated with caps-PS type 19F or caps-PS type 3. Cultures were harvested after 3–5 days of incubation at 37°C in a humidified atmosphere with 5% CO2. Ab production was measured using an ELISPOT assay (see later in text). When indicated, CD4+ and/or CD8+ cells were removed using Dynabeads, or MR1 was added.

ELISPOT assay

Cultured cells were assayed after 3–5 days for the presence of anti-caps-PS Ab-producing cells in an ELISPOT assay as previously described (31, 32). The culture system as described above allows only generation of IgM-producing cells (33, 34). Flexible 96-well ELISA plates were coated overnight with PS 19F or PS 3 in 0.9% NaCl. Thereafter, plates were blocked with 1% BSA in PBS/0.05% Tween 20. Serial dilutions of cultured cells were added to the wells and incubated for 2–4 h at 37°C with 5% CO2 and 100% relative humidity. After removal of the cells, wells were incubated with alkaline phosphatase-conjugated goat anti-mouse IgG for 2 h at 37°C. After washing, spots were visualized by incubation with 5-bromo-4-chloro-3-indolyl phosphate substrate in an agarose gel. After gelling, the plates were incubated for 1 h at 37°C. Spots were counted under a dissection microscope and expressed as spots per 106 cells. Each spot represented an Ab-secreting cell.

Transferring spleen cells from BALB/c mice to SCID mice

BALB/c mice were sacrificed under ether anesthesia and the spleen was removed. Mouse mononuclear cells were isolated from the spleen (see above). When indicated, CD4+ and/or CD8+ lymphocytes were removed using Dynabeads. The percentage of B lymphocytes, CD4+ and CD8+ T lymphocytes in the lymphocyte population was determined by flow cytometry (FACScan, BD Biosciences). The cells were diluted in 500 μl 0.9% NaCl and incubated into SCID mice. On the same day, 500 μg of MR1 was administered and the next day, Pneumovax was given i.p. After 14 days, blood was drawn by heart puncture and IgM and IgG Abs to caps-PS were measured.

Approval of the study was granted by the local committee of the Catholic University Leuven.

Immunization of BALB/c mice

BALB/c mice were immunized with Pneumovax. The vaccine was 1/25 diluted in 0.9% NaCl. Five hundred microliters of this diluted vaccine was given i.p. After 7 and 14 days, blood was drawn by intracardial puncture and anti-caps-PS Abs were detected by ELISA (see later in text). In experiments in which the effects of MR1 were studied, 500 μg of MR1 was injected i.p. 1 day before immunization with Pneumovax. Five hundred microliter of the hamster IgG control Ab was injected i.p. into the control group.

ELISA for detection of anti-caps-PS Abs

A Covalink ELISA 96-well plate was coated overnight at 37°C with pneumococcal polysaccharide (final concentration, 3 μg/ml in N-(3-dimethylaminopropyl)-N′-ethy carbamimide hydrochloride). After coating, the plate was washed three times with 0.05% Tween 20 in PBS. Thereafter, the plate was blocked for 1 h at 37°C with 10% goat serum in PBS. Serum was treated with pneumococcal C-polysaccharide to remove anti-C-polysaccharide Abs. Such treatment consisted of incubation of the serum with a 1/4 dilution of C-PS for 1 h at 37°C. Serial dilutions of serum were made in 0.9% NaCl, 5% glucose, and water. Five hundred microliters of this diluted serum was added to the wells and incubated for 2 h at 37°C. After washing three times with 0.05% Tween 20 in PBS, peroxidase-conjugated goat anti-mouse IgM or goat anti-mouse IgG in a dilution of 1/5000 was added to the wells. The plate was incubated for 1.5 h at 37°C. Thereafter, 3,3′,5′-tetramethylbenzidine was added for color development. After 15 min, the reaction was stopped by an acid stock solution that contained 1 N HCl and 3 N H2SO4. Plates were read at 450 nm.
Results

In vivo effect of blocking CD40L on the Ab response to pneumococcal caps-PS

To address the question whether CD40-CD40L interactions are necessary for the generation of an Ab response to pneumococcal caps-PS in vivo, the effect of anti-CD40L treatment on mice immunized with Pneumovax was studied. Mice immunized with Pneumovax were treated with either MR1 or with a control hamster IgG Ab (control group). MR1 administration was done 1 day before immunization with the vaccine. The IgM as well as the IgG Ab response to various polysaccharide types was determined 14 days after immunization. The results are shown in Figs. 1 (for IgM) and 2 (for IgG). Anti-CD40L treatment inhibited the primary IgM Ab response to all caps-PS types tested (3, 4, 14, and 19F). As has been previously demonstrated, the magnitude of the IgG response was dependent on the serotype (35). There was a high immune response to caps-PS type 3, an intermediate immune response to caps-PS type 14, and almost no IgG immune response to caps-PS type 19F. Immunization of mice with Pneumovax resulted in significantly variable IgG responses to caps-PS type 4. MR1 unmistakably inhibited the high anti-caps-PS type 3 immune response and the intermediate anti-caps-PS type 14 response. MR1 did not affect the low anti-caps-PS 19 IgG immune response. Neither did it have an effect on the rather unpredictable immune response to caps-PS 4.

Taken together, these experiments provide direct evidence that blocking CD40L decreased the IgM as well as the IgG Ab response to various serotypes of caps-PS, measured 14 days after immunization with Pneumovax.

In vitro effect of blocking CD40L on the IgM Ab response to pneumococcal caps-PS type 19F and type 3

The above described experiments showed that anti-CD40L mAbs inhibited the Ab response to pneumococcal capsular Ags in vivo. To investigate whether this inhibitory effect could also be demonstrated in vitro, spleen cells obtained from BALB/c mice were cultured and IgM-specific Abs to caps-PS type 3 and type 19F were measured by ELISPOT assay. Cell cultures in the presence of caps-PS elicited a clear Ab response (Fig. 3). Addition of MR1 markedly decreased the number of anti-caps-PS IgM Ab-secreting cells for caps-PS type 3 as well as for caps-PS type 19F. This inhibitory effect was dependent on the concentration of the mAb and was observed with MR1 concentrations between 10 and 40 μg/ml (data not shown). Addition of hamster IgG did not affect the caps-PS-specific immune response to PS19F and only slightly inhibited the Ab response to caps-PS type 3. These studies show that

![Figure 1](http://www.jimmunol.org/Downloadedfrom/TheJournalofImmunology2775)
blocking CD40L inhibited the in vitro Ab response to caps-PS type 3 and type 19F.

**In vivo effect of blocking CD40L on the augmenting effect of CD4+/H11001 T lymphocytes on the Ab response to pneumococcal caps-PS**

The previous experiments show that MR1 interfered with the development of the Ab response to caps-PS. However, the mechanism by which anti-CD40L (MR1) treatment suppressed the humoral immune response to polysaccharide Ags was not clear. A series of experiments was performed to explore the possibility that MR1 inhibited the anti-caps-PS immune response by blocking the CD40-CD40L interaction between B cells and CD4+ T lymphocytes.

To address this question, transfer experiments were performed in which a B lymphocyte fraction or a B lymphocyte fraction with CD4+ T lymphocytes were transferred to scid/scid mice. After reconstitution of the scid/scid mice with either B lymphocytes or B and CD4+ T lymphocytes, Pneumovax was given and the effect of MR1 on the IgM Ab formation to caps-PS type 3, 4, 6B, and 19F was studied. scid/scid mice that received the B cell fraction and Pneumovax only generated a small Ab response against caps-PS (Fig. 4). When B lymphocytes and CD4+ T lymphocytes were transferred into scid/scid mice, an increase in the IgM Ab titer to the four serotypes was observed when compared with the condition in which only a B lymphocyte fraction was transferred. Administration of MR1 inhibited the CD4+ T lymphocyte-stimulated IgM Ab response (Fig. 4).

Transfer experiments in which mice lymphocytes were transplanted to scid/scid mice resulted in very weak IgG Ab responses and did not allow us to reproducibly study the IgG immune response to caps-PS Ags.

**In vivo effect of blocking CD40L on the suppressive effect of CD8+/H11001 T lymphocytes on the Ab response to pneumococcal caps-PS**

In contrast to CD4+/H11001 T lymphocytes, which have a stimulatory effect on the anti-caps-PS immune response, CD8+/H11001 T lymphocytes have been reported to inhibit the anti-caps-PS immune response (36). Considering the fact that CD8+ cells might also express CD40L, we examined whether blocking the CD40-CD40L interaction would affect the inhibitory effect of CD8+ cells on the caps-PS immune response (37, 38).

Reconstitution of scid/scid mice with murine spleen cells from which the CD8+ T lymphocyte fraction had been removed resulted in an enhanced IgM immune response to caps-PS type 3 when transferred into scid/scid mice, an increase in the IgM Ab titer to the four serotypes was observed when compared with the condition in which only a B lymphocyte fraction was transferred. Administration of MR1 inhibited the CD4+ T lymphocyte-stimulated IgM Ab response (Fig. 4).

Transfer experiments in which mice lymphocytes were transplanted to scid/scid mice resulted in very weak IgG Ab responses and did not allow us to reproducibly study the IgG immune response to caps-PS Ags.
compared with the immune response observed in SCID mice reconstituted with total murine spleen cells (Fig. 5). These data confirm that CD8\(^+\)/H11001 T lymphocytes have an inhibitory effect on the murine immune response to pneumococcal caps-PS.

To study whether the inhibitory effect of CD8\(^+\) T lymphocytes on the anti-caps-PS immune response was mediated through CD40-CD40L interaction, murine spleen cells from which the CD4\(^+\)/H11001 T lymphocyte fraction had been removed were transferred...
to scid/scid mice, and the Ab response to Pneumovax was measured in the presence or the absence of MR1. The IgG Ab response was very weak and could not be measured. Results are shown in Fig. 6. Transfer of CD4+ T lymphocyte-depleted murine spleen cells to scid/scid mice elicited only a weak Ab response. Addition of MR1 consistently tended to increase the immune response to caps-PS type 3 (p = 0.066) and significantly increased the immune response to caps-PS type 4. There was no effect on the very weak immune response to caps-PS type 19F.

In vitro effect of blocking CD40L on the effect of CD4+ and CD8+ T lymphocytes on the Ab response to pneumococcal caps-PS

Finally, the effect of MR1 on the in vitro IgM Ab formation to caps-PS type 3 and 19F by total murine spleen cells or murine spleen cells from which CD4+ and/or CD8+ T lymphocytes had been removed was studied. The results are shown in Fig. 7. Murine spleen cells from which CD8+ T lymphocytes had been removed mounted a higher Ab response than murine spleen cells from which both CD4+ and CD8+ T lymphocytes had been removed. This provided evidence that CD4+ T lymphocytes amplify the anti-caps-PS Ab response in vitro. Addition of MR1 to the CD8+ T lymphocyte-depleted murine spleen cells markedly decreased the Ab response, showing that the amplifying effect of CD4+ T lymphocytes is CD40/CD40L dependent. These effects were observed for the immune response to caps-PS type 19F as well as for the immune response to caps-PS type 3.

In contrast to our in vivo observations, we found no increased in vitro Ab response by CD8+ T lymphocyte-depleted murine spleen cells when compared with the immune response by total

![Figure 5](http://www.jimmunol.org/)

**FIGURE 5.** In vivo effect of CD8+ T lymphocyte depletion on the IgM Ab response to pneumococcal caps-PS. SCID/SCID mice were reconstituted with murine spleen cells or with murine spleen cells depleted of CD8+ T lymphocytes. Total murine spleen cells consisted of 51–57% B lymphocytes, 33–31% CD4+ T lymphocytes, and 9–15% CD8+ T lymphocytes. Depletion of only the CD8+ T lymphocyte fraction resulted in a cell population consisting of mainly B lymphocytes (53–58%) and CD4+ T lymphocytes (24–32%) with <1.5% CD8+ T lymphocytes. The cells were suspended in 0.9% NaCl and injected i.p. into SCID/SCID mice. The total amount of B lymphocytes was the same for all conditions and amounted to 10 ± 10^6. The IgM and IgG immune response to caps-PS type 3 was measured after 14 days (CD8+ T lymphocyte-depleted murine spleen cells plus Pneumovax; ▲, murine spleen cells plus Pneumovax). Results shown are means ± 1 SD of three independent experiments. * Statistically significantly different (p < 0.05) by Wilcoxon-Mann-Whitney U test.

![Figure 6](http://www.jimmunol.org/)

**FIGURE 6.** In vivo effect of MR1 on CD4+ T lymphocyte depletion on the immune response to caps-PS. SCID/SCID mice were reconstituted with murine spleen cells depleted of CD4+ T lymphocytes (●), □, in the presence (■) or absence (○) of MR1. Total murine spleen cells consisted of 52–56% B lymphocytes, 24–32% CD4+ T lymphocytes, and 9–15% CD8+ T lymphocytes. Depletion of the CD4+ T lymphocytes resulted in a cell population consisting of mainly B lymphocytes (62–69%) and CD8+ T lymphocytes (15–26%) with <1.5% CD4+ T lymphocytes. The cells were suspended in 0.9% NaCl and injected i.p. into SCID/SCID mice. The total amount of B lymphocytes was the same for all conditions and amounted to 9.5 ± 10^6. The IgM immune response to caps-PS 3 and caps-PS 4 was measured in duplicate after 14 days ( Errors in murine spleen cells – CD4+ T lymphocytes plus Pneumovax; ■, murine spleen cells – CD4+ T lymphocytes plus MR1). Results shown are mean ± 1 SD of three independent experiments. * Statistically significantly different (p < 0.05) by Wilcoxon-Mann-Whitney U test.
murine spleen cells. CD4+ T lymphocyte-depleted murine spleen cells mounted a decreased immune response when compared with the Ab response elicited by total murine spleen cells. Addition of MR1 to CD4+ T lymphocyte-depleted murine spleen cells resulted in a significantly increased Ab response to caps-PS 3 and consistently tended to augment the anti-caps-PS type 19F Ab response.

Discussion

Accurate determination of protective immune responses to pneumococcal vaccination requires assay of caps-PS-specific Abs. Although pneumococcal cell wall polysaccharide (C-PS) are highly immunogenic, Abs against C-PS are not protective (4, 5). Commercial pneumococcal vaccines are known to be contaminated with up to 50% C-PS and thus will induce anti-C-PS Abs. Therefore, it is of utmost importance to preincubate serum with C-PS to remove anti-C-PS Abs in ELISA methods used to study postvaccination titers. Anti-C-PS Abs have been detected in 99% of the elderly, likely accumulated from past exposures to Streptococcus pneumoniae, S. mitis, and Hemophilus influenzae (39). The polysaccharide capsule is the principal determinant of the virulence of pneumococci. Protection after immunization is dependent upon production of the circulating type-specific anti-caps-PS Abs (40). There have been several reports describing patients having specific Ab deficiency with normal Igs who have normal IgA, IgM, IgG, and IgG subclasses, but who fail to develop a specific Ab response to caps-PS (41, 42). These patients suffer from recurrent infections with encapsulated bacteria and are diagnosed by a failure to respond to vaccination with purified caps-PS (41, 42). There is an unmet clinical need to understand the physiological regulation of the immune response to caps-PS. Such knowledge could be used to develop future therapeutic measures for patients with specific Ab deficiency with normal Igs.

Although the mechanisms underlying the noncognate T lymphocyte modulation of the TI immune response are currently a matter of speculation, it is generally believed that the immune response to purified soluble polysaccharides with or without haptens is independent of the CD40-CD40L interaction (19, 21–23). In the present manuscript, we critically readressed the question whether the CD40-CD40L interaction is essential for the regulation of the anti-caps-PS immune response. All previous studies did not unconnectedly consider the interaction between B lymphocytes and CD4+ T lymphocytes and between B lymphocytes and CD8+ T lymphocytes. By carefully and separately investigating the effect of CD4+ T lymphocytes and CD8+ T lymphocytes on the anti-caps-PS immune response and by using MR1, an antagonist mAb against CD40L, we were able to unambiguously demonstrate that the noncognate T lymphocyte modulation of the TI immune response is markedly dependent on CD40L interactions. This interaction not only plays a pivotal role for the IgG response but also for the IgM response.

Our in vitro and in vivo studies illustrated that CD4+ T lymphocytes provide help to the immune response to caps-PS and that this helper function is dependent on CD40L interactions. B lymphocytes in the presence of CD4+ T lymphocytes mounted a notably higher specific immune response to caps-PS than B lymphocytes in the absence of CD4+ lymphocytes. CD8+ T lymphocytes, on the other hand, had a suppressor effect on the immune response to caps-PS. This was demonstrated in vivo by the increased immune response after CD8+ T lymphocyte depletion. CD4+ T lymphocyte-depleted murine spleen cells, leaving a B and CD8+ T lymphocyte fraction, elicited only a weak in vivo and in vitro Ab response. Addition of MR1 to such CD4+ T lymphocyte-depleted murine spleen cells resulted in an increased Ab response. The effect of MR1 was mediated through CD8+ T lymphocytes since MR1 exerted no effect on the immune response generated by CD4+ and CD8+ T lymphocyte-depleted murine cells (data not shown).

Collectively, these data provide evidence that CD8+ T lymphocytes inhibit the anti-caps-PS Ab response and support the idea that blocking CD40L on CD8+ T lymphocytes results in opposing the inhibitory effect of CD8+ T lymphocytes on the immune response to anti-caps-PS.

The observation that MR1 did not affect the anti-caps-PS immune response in the absence of CD4+ and CD8+ cells suggested that the effect of MR1 did not depend on the blocking of the CD40-CD40L interaction between B lymphocytes and B lymphocytes, between B lymphocytes and NK cells, or between B lymphocytes and APCs.
Polysaccharides have the capacity to cross-link Ag receptors on the surface of B lymphocytes and induce Ab production. Multivalent membrane cross-linking on the polysaccharide-specific B cell also induces B cell responsiveness to various cytokines and microbial adjuvants for the induction of B cell maturation and Ig class switch. The release of cytokines by T lymphocytes could augment the anti-caps-PS immune response by B cells. We here demonstrate that the CD40-CD40L interaction plays an essential role in the T cell-mediated modulation of the immune response to caps-PS. T cells, once activated, could either directly or indirectly modulate B cell activity via CD40L. Direct ligation of CD40 on B lymphocytes, with subsequent stimulation, is possible. However, an indirect mechanism whereby T lymphocytes interact with APCs cannot be excluded. Contact of T lymphocytes with macrophages and/or dendritic cells through CD40-CD40L interaction could induce the release of multiple cytokines that can further stimulate B lymphocytes.

To our surprise, neutralization of CD40L not only reduced the in vivo IgG immune response, but also resulted in an inhibition of the IgM immune response. A marked inhibitory effect of MR1 on the IgM anti-caps-PS immune response was also demonstrated in vitro. This was unexpected since for T-dependent Ag, CD40-CD40L interaction is important for isotype switch but not for IgM production. Patients with mutations in the CD40L gene do not fail to generate IgM Abs but fail short in producing IgA and IgG Abs. Our observations demonstrate that CD40L interactions are also vital for modulating the IgM response to TI-2 Ags. This might be through a direct effect of T cells on B cells or through an indirect effect, as discussed above.

It is an unresolved question how CD4+ T cells are activated by caps-PS. Polysaccharides do not appear to bind class II MHC molecules (43). One could hypothesize that T cells are triggered by APCs. Through contact with caps-PS, APCs get stimulated and such stimulation could result in the expression of costimulatory molecules and the secretion of various cytokines that can further stimulate T cells. A possible candidate for binding and presenting polysaccharides to T lymphocytes is CD1. This molecule is expressed on dendritic cells and has been described to process and present lipids and glycolipids to T lymphocytes (44, 45).

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