Specific Intracellular Adhesion Molecule-Grabbing Nonintegrin R1
Is Not Involved in the Murine Antibody Response
to Pneumococcal Polysaccharides

Leen Moens,1 Axel Jeurissen,1 Greet Wuyts,1 Padraic G. Fallon,2 Boon Louis,3
Jan L. Ceuppens,4 and Xavier Bossuyt1*

Department of Medical Diagnostic Sciences, Laboratory of Experimental Laboratory Medicine, Faculty of Medicine, Catholic University Leuven, Leuven, Belgium; Department of Biochemistry, Trinity College, Dublin 2, Ireland; Bioceros BV, Yalelaan 46, 3584 CM Utrecht, The Netherlands; and Department of Pathophysiology, Laboratory of Experimental Immunology, Faculty of Medicine, Catholic University Leuven, Leuven, Belgium

Received 20 April 2007/Returned for modification 31 May 2007/Accepted 5 September 2007

Streptococcus pneumoniae is a microorganism that frequently causes serious infections in children, the elderly, and immunocompromised patients. We studied whether the specific intracellular adhesion molecule-grabbing nonintegrin R1 (Sign-R1) receptor, involved in the uptake of capsular polysaccharides (caps-PS) by antigen-presenting cells, is necessary for the antibody response to pneumococcal caps-PS and phosphorylcholine (PC). The antibody response to caps-PS and PC was evaluated after vaccination with soluble caps-PS (Pneumovax) and after vaccination with heat-killed S. pneumoniae. The role of Sign-R1 was investigated by using Sign-R1 knockout mice and anti-Sign-R1 monoclonal antibodies. The immunoglobulin M (IgM) and IgG antibody response to PC and caps-PS (serotypes 3 and 14) was not affected by anti-Sign-R1 monoclonal antibodies. The IgM antibody response in Sign-R1 knockout mice was comparable to the antibody response in wild-type mice. The IgG antibody response to serotype 3, but not to serotype 14, tended to be lower in Sign-R1 knockout mice compared to wild-type mice. In conclusion, we found that Sign-R1 is not involved in the IgM antibody production to PC and caps-PS serotype 3 or 14 and the IgG immune response to PC and caps-PS serotype 14. There is no direct relation between capture and uptake of caps-PS serotype 14 by Sign-R1 and the initiation of the anti-caps-PS antibody production in mice.

Streptococcus pneumoniae is a major human pathogen. Infections with, S. pneumoniae result in substantial morbidity and mortality, particularly in young children, the elderly, and immunocompromised patients (16). In various animal species and in humans, protection against infection with S. pneumoniae is mediated by antibodies against the pneumococcal capsular polysaccharides (caps-PS) (2). Vaccination with isolated pneumococcal caps-PS is widely used to protect people against infection with S. pneumoniae (14).

caps-PS are classified as T-lymphocyte-independent type 2 antigens (15). The induction of a humoral immune response to caps-PS is independent of T lymphocytes, but T lymphocytes influence the antibody response to caps-PS (6–8, 15). There are only scarce data available on the role of antigen-presenting cells (APCs) in the immune response to isolated T-lymphocyte-independent type 2 antigens. Garcia de Vinuesa et al. found that administration to mice of agonistic CD40 monoclonal antibodies (MAbs), together with a polysaccharide antigen, not only enhanced the antibody response but also markedly increased the amount of APCs in the spleen (3). It was hypothesized that CD40 MAbs activate APCs, which then would activate T lymphocytes through cytokine secretion (3). Garg et al. showed that, in contrast to in vitro culture of spleen cells, in vitro culture of lymph node cells did not respond to caps-PS and that the addition of APCs isolated from spleen cells enabled the lymph node to respond to caps-PS (4). It was further put forward that defects in APC function might play a critical role in the failure of neonates to respond to caps-PS (1). These data suggest that APCs play a role in the immune response to isolated caps-PS antigens.

In the present study we addressed the question of whether specific intracellular adhesion molecule-grabbing nonintegrin R1 (Sign-R1) is involved in the antibody response to caps-PS. Sign-R1 is a C-lectin that contributes to the uptake of caps-PS by macrophages (5, 18). Sign-R1 is expressed on marginal zone macrophages in the spleen, on medullar and subcapsular macrophages in lymph nodes (5), and on resident peritoneal macrophages (19). It is necessary for the uptake and endocytic internalization of polysaccharides, such as neutral and anodic forms of dextran (with a wide variety of molecular masses [70 to 2,000 kDa]) and Ficoll (10). Sign-R1 also captures encapsulated S. pneumoniae (serotypes 3 and 14) and soluble caps-PS (described for serotypes 14, 23, and 26) (9). The administration of anti-Sign-R1 antibodies inhibited the Sign-R1-mediated uptake of caps-PS or dextrans (9). Taken together, Sign-R1 is considered an important pathogen recognition receptor for uptake and clearance of blood-born antigens in vivo (5). In contrast to wild-type mice, Sign-R1 knockout mice showed increased mortality after intraperitoneal infection with S. pneumoniae (13). It has been suggested that Sign-R1 con-
tributed to protection against pneumococcal infection in mice by clearing the bacteria (9). In contrast to wild-type mice, the knockout mice displayed severely enhanced inflammatory parameters and failed to produce a rapid immunoglobulin M (IgM) anti-phosphorylcholine (anti-PC) response. It was suggested by Koppel et al. (12) that S. pneumoniae was captured by Sign-R1 on marginal zone macrophages for antigen presentation and activation of marginal zone B cells, resulting in an IgM anti-PC response. Lanoue et al. (13), on the other hand, suggested that Sign-R1 contributed to protection against pneumococcal infection in mice by clearing the bacteria and not by reducing the natural IgM anti-PC antibody levels.

In the present study, we investigated whether Sign-R1 is involved in the antibody response to pneumococcal caps-PS and PC.

MATERIALS AND METHODS

Materials. Pneumovax, a 23-valent pneumococcal vaccine, was obtained from Aventis Pasteur MSD, Belgium. Pneumococcal caps-PS were obtained from ATCC, Rockville, MD. C-polysaccharide was obtained from Statens Serum Institute, Denmark. NaCl 0.9% was from Vaccumed, Ghent, Belgium. Covalink and MaxiSorp ELISA 96-well plates were obtained from Naige Nunc International, Denmark. Tween 20 was obtained from Sigma-Aldrich, N.V./S.A., Bornem, Belgium. Phosphate-buffered saline (PBS) and goat serum were from Gibco-BRL/Life Technologies, Ltd., Paisley, Scotland. Peroxidase-conjugated goat anti-mouse IgM and IgG were from Nordic Immunological Laboratories, Aventis Pasteur MSD, Belgium. Pneumococcal caps-PS were obtained from Merck KgaA, Darmstadt, Germany. Isoflurane was obtained by Schering-Plough Animal Health, Harelbeke, Middlesex, United Kingdom. HEPES solution was from Merck KgaA, Darmstadt, Germany. Isoflurane was isolated from Covington-Prophage Animal Health, Harelbeke, Middlesex, United Kingdom. HEPES solution was from Merck KgaA, Darmstadt, Germany.

Mice. BALB/c and C57BL/6 mice were bought at Elevage, Janvier, France. Sign-R1 knockout mice and control C57BL/6 mice were from N. McKenzie (Medical Research Council, Cambridge, United Kingdom) and P. G. Fallon (Trinity College, Dublin, Ireland). The animals were used at age 6 to 8 weeks and were kept under a standard protocol with free access to pelleted food and water. Approval for the study was granted by the local ethics committee of the Catholic University Leuven.

Immunofluorescence analysis. The in vivo FITC-dextran capture and the inhibition of the FITC-dextran capture by Sign-R1 was confirmed by immunofluorescence analysis as previously described (5).

Immunization of mice. Mice were immunized with Pneumovax or with heat-killed S. pneumoniae serotype 3 or serotype 14. The vaccine was 1/25 diluted in NaCl 0.9%. A total of 500 µl of this diluted vaccine was given intraperitoneally (i.p.). For immunization with S. pneumoniae, 2 × 10^8 CFU in 200 µl of PBS were injected i.p. After 14 days, blood was drawn by intracardiac puncture in iso- fluorane-anesthetized mice. Mice were euthanized after iso- fluorane inhalation by cervical dislocation. IgM and IgG anti-PC and anti-caps-PS antibodies were detected by enzyme-linked immunosorbent assay (ELISA) (see below). In the experiments in which transient Sign-R1 knockout mice were developed, 200 µg of 22D1 or 2 µg of ER-TR9 was given i.p. In one experiment, 100 µg of 22D1 was given intravenously (i.v.) into the tail vein 1 day before immunization. The control group was injected with 200 µg of hamster IgG i.p., 2 mg of rat IgM i.p., or 100 µg of hamster IgG i.v.

IgM and IgG anti-PC and anti-caps-PS ELISA. Anti-PC antibodies were measured as previously described (20). Anti-caps-PS antibodies were measured as previously described (7). Serum was treated at room temperature for minimum 30 min with pneumococcal C-polysaccharide (5 µg of PBS/ml, 2% goat serum) and caps-PS serotype 22F (5 µg of PBS/ml, 2% goat serum) to remove anti-C-polysaccharide antibodies and non-S. pneumoniae-specific antibodies, respectively.

Statistical analysis. Differences in antibody levels were evaluated by use of the Mann-Whitney U test. A P value of <0.05 was considered significant.

RESULTS AND DISCUSSION

The role of Sign-R1 in the anti-PC and anti-caps-PS antibody production was evaluated by using the anti-Sign-R1 MAbs 22D1 and ER-TR9. The ability of both MAbs to block Sign-R1 was confirmed by immunofluorescence analysis (5).

The IgM and IgG anti-PC antibody levels were measured after immunization with heat-inactivated S. pneumoniae (serotypes 3 and 14). The IgM anti-PC antibody production was already high at day 4 and peaked between days 7 and 10 after immunization. The IgG anti-PC antibody production displayed similar kinetics, but the antibody levels were much lower than the antibody levels for IgM (data not shown). The IgM and IgG anti-PC antibody production in mice treated with anti-Sign-R1 antibodies (ER-TR9 or 22D1) was comparable to the IgM and IgG anti-PC antibody production in mice treated with control antibodies. The IgM anti-PC antibody production at different time points is shown in Fig. 2.

In various animal species and in humans, protection against infection with S. pneumoniae is, in addition to natural anti-PC IgM antibodies, mediated by antibodies against the pneumococcal caps-PS (2). In view of the contradictory data with

FIG. 1. Inhibition of in vivo FITC-dextran capture by anti-SignR-1 MAbs. BALB/c mice were, prior to i.v. injection with FITC-dextran (1 mg/ml), treated with rat IgG (5 µg/ml) (a), 22D1 (5 µg/ml) (b), and ER-TR9 (5 µg/ml) (c) by i.v. injection. Spleens were isolated, and immunofluorescence analysis of spleen tissue sections was performed.
regard to the role of Sign-R1 in the anti-PC antibody production, we investigated the role of Sign-R1 in the IgM and IgG antibody production to caps-PS. Two complementary approaches were used: monoclonal anti-Sign-R1 antibodies and Sign-R1 knockout mice. In a first approach to evaluate the role of Sign-R1 in the anti-caps-PS IgM and IgG antibody response, we studied the effect of administering anti-Sign-R1 MAb. BALB/c mice were immunized with Pneumovax in the presence or absence of ER-TR9 or 22D1. The antibody response to serotypes 3 and 14 was measured after 14 days. The results of the 22D1 treatment are shown in Fig. 3 and indicate that the antibody response in the animals that received anti-Sign-R1 was similar to the antibody response in animals that received a control antibody. Likewise, ER-TR9 treatment did not affect the anti-caps-PS antibody response (data not shown). Administration of a double dose of 22D1 antibody (1 day before vaccination and 6 days after vaccination), in order to ensure prolonged blocking of Sign-R1 (9), did not affect the anti-caps-PS antibody response (data not shown). We also tested the effect of 22D1 administration in C57BL/6 mice and found that such treatment did not affect the anti-caps-PS immune response (six mice/group [data not shown]).

Similarly, i.v. administration of anti-Sign-R1 did not affect the anti-caps-PS antibody response (data not shown). It has been suggested by Kang et al. that i.p. administration of anti-Sign-R1 MAb primarily eliminated Sign-R1 from the lymph nodes, whereas i.v. administration downregulated Sign-R1 expression in the spleen (9).

Finally, we tested whether anti-Sign-R1 treatment affected the antibody response to caps-PS after immunization with intact heat-inactivated bacteria. BALB/c mice were immunized with heat-inactivated S. pneumoniae on day 0 and treated with anti-Sign-R1 (n = 6 [□]) or rat IgG (n = 6 [□]) on day 1. The IgM and IgG antibody responses to pneumococcal caps-PS serotypes 3 and 14 were measured 14 days after immunization. The results (means ± the standard errors of the mean) show the absorbance values at various serum dilutions and are representative of two independent experiments. P > 0.12, P > 0.29, P > 0.28, and P > 0.49 for the IgM results on days 4, 7, 10, and 14, respectively.

In a second approach, the anti-caps-PS antibody response in Sign-R1 knockout mice was compared to the antibody response in wild-type mice. The results are shown in Fig. 4. The IgM and IgG response in Sign-R1 knockout mice was comparable to the IgM and IgG response in the wild-type mice for serotype 14. The IgG antibody response to serotype 3 tended to be lower in Sign-R1 knockout mice compared to the C57BL/6 wild-type mice. The IgM response in Sign-R1 knockout mice was comparable to the IgM response in the C57BL/6 wild-type mice for serotype 3.

We found that anti-Sign-R1 treatment did not affect the IgM and IgG anti-PC and anti-caps-PS antibody response for caps-PS serotype 3 and caps-PS serotype 14. Similarly, the IgM and IgG antibody response to caps-PS serotype 14 in Sign-R1 knockout mice was comparable to the antibody response in wild-type mice. For caps-PS serotype 3 the IgM response in
Sign-R1 knockout mice was comparable to the antibody response in wild-type mice. The IgG response tended to be lower in Sign-R1 knockout mice compared to wild-type mice. Our data thus indicate that the Sign-R1 pathway is not involved in the antibody response to pneumococcal caps-PS. Other pathways must be implicated. Sen et al. (17) reported that IgG anti-caps-PS serotype 3 and anti-caps-PS serotype 14 was diminished in Toll-like receptor 2 (TLR-2/H11002/H11002/H11002) mice compared to wild-type mice. This finding might be attributed to the fact that the pneumococcal vaccine contains TLR-2 and TLR-4 ligands that stimulate the IgG response. Kobrynski et al. (11) suggested that CD1, a major histocompatibility complex class I-like molecule expressed on the surface of immature thymocytes, dendritic cells, activated macrophages, and B lymphocytes, is required for the production of IgG anti-caps-PS antibodies (caps-PS serotype 4 and caps-PS serotype 19). These researchers postulated that caps-PS are presented by CD1 molecules expressed on APCs and B cells. CD1-expressing APCs and B cells can elicit helper functions from CD1-responsive effector T cells.

In summary, we found that Sign-R1 is not involved in the IgM and IgG anti-PC antibody production. Moreover, Sign-R1 is also not involved in the IgM antibody production to caps-PS serotype 3 and caps-PS serotype 14, and the IgG immune response to caps-PS serotype 14. There is no direct relation between capture and uptake of caps-PS serotype 14 by SignR-1 and the initiation of IgM and IgG anti-caps-PS serotype 14 production in mice.

ACKNOWLEDGMENTS

This study was supported by grants of the Catholic University Leuven (Onderzoekstoelage) and the FWO (Fund for Scientific Research–Vlaanderen). X.B. is a senior clinical investigator of the FWO-Vlaanderen.

REFERENCES


