Cellular Immune Responses during Gonococcal and Meningococcal Infections

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The genus *Neisseria* contains two species of clinical importance: *Neisseria gonorrhoeae* and *Neisseria meningitidis*. Although these two microorganisms are closely related genetically, the diseases they produce differ greatly. *N. gonorrhoeae* is the etiologic agent of gonorrhea, currently among the most common of the classical sexually transmitted diseases, whereas *N. meningitidis* is the causative agent of meningococcal meningitis. Although these *Neisseria* species have been known for over a century and there are effective antibiotic therapies for both of these diseases, the morbidity and mortality associated with them remain significant. For example, the pediatric mortality of meningococcal infection places it among the top 10 leading causes of death of children in North America. Similarly, the number of cases of gonorrhea reported to the Centers for Disease Control has averaged about three-quarters of a million per year for the past several years.

Despite a number of studies, the immunological mechanisms responsible for providing antigonococcal protection have been completely elucidated. In part, this is due to the lack of an animal model for studying gonococcal infection. Equally important, however, is the fact that most studies have concentrated on the systemic immune response to gonococcal infection (19, 25, 32, 37, 38), whereas the initiation of the infection occurs locally in a site apparently isolated from these circulating immune modalities. Thus, to completely understand antigonococcal immunity, it is necessary to study the local immune responses available at or near mucosal surfaces in the male and female reproductive tracts.

Serum antibodies specific for a variety of surface antigens have been described in both meningococcal and gonococcal infections, and in some cases the presence of these antibodies can be correlated with resistance to infection. It has been proposed that these antibodies could be involved in complement-mediated bacteriolysis or opsonization of the microorganisms or both. Although these mechanisms may be important in providing systemic immunity, sites where the *Neisseria* spp. are commonly found (the nasopharynx and reproductive tract) are sparsely populated with phagocytes and are deficient in many of the components of complement.

Recently, an increasing number of reports suggest that beside macrophage-dependent activities, cell-mediated responses such as cytotoxic T lymphocytes, natural killer (NK) cells, and antibody-dependent cell-mediated cytotoxicity (ADCC) might also have a role in the host defense against bacterial infection. A number of bacteria, including both gonococci and meningococci, make initial contact with the host at the mucosal level. It would follow that immunosurveillance mechanisms should be especially active at these anatomical sites. It is striking that large granular lymphocytes, the main effector cells of NK cell activity and ADCC,

are present in large numbers in the epithelium and the lamina propria of mucosal tissue and are able to exert their functional activity at this level. NK cells and ADCC appear to be manifestations of different functions of the same lymphocyte, and it has been suggested that intestinal large granular lymphocytes may play a role in the antibacterial secretory immunoglobulin A (sIgA)-dependent ADCC (13). A second, well characterized class of lymphocytes, the cytotoxic T lymphocytes, also may exist in mucosal sites and may be active in providing antibacterial immunity. Most of these studies of cytotoxic mechanisms have focused on one or another of these distinct cell types, which have clear differences in the way they recognize target cells. It is the purpose of this paper to review recent evidence on cell-mediated immune (CMI) responses to pathogenic *Neisseria* spp. and to reevaluate their role in the development of antibacterial immunity.

HOST CELLULAR DEFENSES IN GONOCOCCAL INFECTION

CMI Response to Gonococcal Infections

A few early reports in the literature suggest that a CMI response occurs in patients with gonococcal infection (1, 2, 5, 12, 31). An initial report of a delayed-type hypersensitivity response to gonococcal infection was published by Teague and Torrey (36). During the 1930s and 1940s, delayed-type hypersensitivity was demonstrated in individuals infected with gonococci by using a variety of cellular fractions and culture filtrates (1, 2, 5). Much of this work was done with the intent of developing a better method to diagnose the disease. Results of these studies indicated that most infected patients gave a positive result and up to 86% of normal individuals had a negative response (5). In the 1970s, interest was generated in the CMI response to gonococcal infection. Reports of lymphocyte transformation in gonorrhea (7, 11, 17) leave little doubt of the existence of a CMI response in gonococcal infection. However, a number of aspects of the gonococcal CMI response need clarification. Wyle et al. (39) reported a study in which peripheral blood lymphocyte (PBL) transformation was stimulated by both gonococcal and meningococcal antigens in men and women with uncomplicated gonorrhea. The blastogenic responses of PBLs from these individuals were substantially higher than those of normal controls. This demonstrates cross-reactivity between *N. gonorrhoeae* and *N. meningitidis*. The extent of the blastogenic response in women was much greater than in men. Partial purification of these antigens by gel chromatography resulted in reduced cross-reactive responses to the semipurified meningococcal antigen. Female patients demonstrated marked stimulation with the purified gonococcal antigen, whereas male patients showed slight stimulation with purified gonococcal antigen. Therefore, these authors

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speculated that CMI may act to limit the spread of gonococcal infection beyond the genital mucous membranes.

Assays which are thought to be in vitro correlates of delayed-type hypersensitivity reactions (lymphocyte activation and production of migration inhibition factor) have been used in a variety of studies of human responses to an array of gonococcal products. Kraus et al. (17), in an attempt to determine whether cell-mediated hypersensitivity develops during a naturally acquired gonococcal infection, cultured lymphocytes from infected individuals with a crude sonic extract of gonococci. The lymphocyte response was quantitated by uptake of labeled deoxyribonucleic acid precursors. Lymphocyte transformation induced by gonococcal antigens occurred in PBLs from some men with gonococcal urethritis. Although transformation was not always demonstrable during the initial infection, it was usually present in patients with two or more episodes of infection. The degree of transformation of the lymphocytes from infected patients with the first episode of gonorrhea did not differ significantly from that observed in noninfected patients. It was suggested that the interval between infection and treatment may have been too short for the patients to develop a significant degree of hypersensitivity. In cultures that were positive for gonococcus-induced proliferation, the maximal stimulation occurred after 5 to 6 days of culture. Esquenazi and Streitfeld (7) confirmed that lymphocyte transformation to gonococcal and to *N. catarrhalis* (now *Branhamella catarrhalis*) sonic extracts could be demonstrated. Lymphocytes from infected patients manifested significant uptake of tritiated thymidine in response to one or more antigens. At 5 weeks posttherapy, lymphocytes were relatively nonreactive to gonococcal antigenic stimulation. Blastogenesis in response to *B. catarrhalis* antigens was also seen in a few gonorrhea patients and in normal controls. Cross-reactivity of *B. catarrhalis* and *N. gonorrhoeae* antigens was indicated by the disappearance of reactivity to the *B. catarrhalis* sonic extract by the lymphocytes of almost all convalescent gonorrhea patients. Grimble and McIlmurray (11), using a crude gonococcal antigen, found positive lymphocyte stimulation in about 85% of patients with gonorrhea. However, using antigens prepared from *N. meningitidis*, they were unable to demonstrate a cross-reactive response in individuals with gonococcal infection. Although these reports clearly indicate that a CMI response occurs in gonococcal infection, this response cannot be correlated with protection from gonococcal infection. In a series of studies of Swedish individuals infected with gonorrhea, Rosenthal and Sandstrom (28-30) found no demonstrable differences in the lymphocyte response to gonococcal antigen in 42 patients with gonococcal urethritis and 18 uninfected controls. One explanation for these results could be the presence of asymptomatic male and female carriers of gonococcal infection in the control group. A second explanation is that the systemic response, as measured by the activity of PBLs, does not reflect what is happening locally. A significant difference in lymphocyte reactivity was noted only between female patients and controls.

ADCC and NK Cell Antigonococcal Activity

Floyd-Reising et al. (8) demonstrated that human PBLs can participate in ADCC against *N. gonorrhoeae*. Acute-phase serum samples from individuals with histories of multiple uncomplicated gonococcal infection, in cooperation with human PBLs, are capable of killing various gonococcal isolates in the absence of complement. Both homologous

and heterologous isolates are susceptible to ADCC-mediated antigenococcal activity, with heterologous pelvic inflammatory disease isolates being the most susceptible. In addition to ADCC, unseparated mononuclear cells were capable of natural antigenococcal activity. When the nonimmune mononuclear cells were purified and assayed for their antigenococcal activity, it was demonstrated that adherent monocytes were also capable of a natural antigenococcal activity in the absence of antibody. Inhibition of monocyte phagocytosis by cytochalasin B abolished the natural antigenococcal activity but did not significantly reduce the ADCC-mediated antigenococcal activity. Nonadherent cell populations expressing surface antigens for B, T, or NK cells were not capable of ADCC-mediated antigenococcal activity. However, nonadherent cell populations with either T or NK cell surface markers expressed natural antigenococcal activity. These results indicate that both natural and ADCC-mediated cytotoxicity can be effective against gonococci.

Lymphoid cells of the human fallopian tube have been characterized for cell surface markers and for their participation in ADCC-mediated and natural cytotoxicity. Cooper et al. (3, 4), using discontinuous Percoll gradients to separate fallopian tube lymphoid cells, demonstrated the presence of cells expressing B, T, and NK cell surface markers. The ratio of B to T cells was approximately 1:4. These ratios are consistent with other mucosal sites. Lymphoid-cell populations from the human fallopian tubes were purified over Percoll gradients and used as effector cells against gonococci. A cell fraction ($\rho = 1.076$) from these gradients contained lymphoid cells which were capable of both ADCC-mediated and natural cytotoxicity. PBLs were purified by using plastic adherence and B- and T-cell panning with monoclonal antibodies; these cells were also used as effector cells against gonococci. Populations of T cells contaminated with NK cells were effective in ADCC assays and in natural cytotoxicity. Cytotoxic/suppressor cells ($CD3^+$, $CD8^+$) of high purity were able to express natural cytotoxicity but not ADCC.

Moticka et al. (E. J. Moticka, K. Elliott, T. Hindman, and M. D. Cooper, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988; E51, p. 117) described a suppression of phytohemagglutinin-induced mitogenesis by human fallopian tube lymphoid cells (FTLs). Under a variety of experimental conditions, FTLs responded minimally or not at all to several mitogens including phytohemagglutinin, concanavalin A, pokeweed mitogen, and staphylococcal protein A. Studies were performed to determine whether this lack of mitogenic responsiveness was due to the activity of suppressor cells. FTLs were mixed with allogenic PBLs and cultured with the T-cell mitogen phytohemagglutinin under conditions optimal for the stimulation of PBLs. Culturing the PBLs and FTLs in 1:1 or 2:1 ratios depressed the incorporation of [3H]thymidine by more than 50%. As few as 10% FTLs in cultures of PBLs produced consistent suppression of phytohemagglutinin-induced mitogenesis. Control cultures with similar numbers of allogenic PBLs demonstrated no such suppression, indicating that the effect was not due to allogenic interactions. The suppression was attributable to a soluble factor which could interfere with the reactivity of PBLs. The possibility that suppressor cells and suppressor factors play a role in down-regulating antigenococcal cytotoxic T cells is currently under investigation.

CELLULAR RESPONSES IN ANIMALS IMMUNIZED WITH *N. GONORRHOEAE*

Kannungo and Agarwal (15), using a guinea pig subcutaneous chamber model, studied the development of cellular immunity by using lymphocyte and macrophage migration as measurements of CMI. They infected the subcutaneous chambers of guinea pigs with gonococci. At 1 week postinfection there were indications of CMI, which then persisted for up to 7 weeks. The CMI response was detected by using a crude soluble cellular antigen, crude lipopolysaccharide (LPS), and a ribonucleic acid protein antigen prepared from a local clinical isolate. Koostra et al. (16) studied the CMI of guinea pigs immunized with either gonococcal or meningococcal ribosomes and demonstrated a quantitative but not a qualitative specificity. They found about a fivefold difference in the concentration of *N. gonorrhoeae* and *N. meningitidis* skin test antigens necessary to obtain a positive response to guinea pigs immunized with gonococcal ribosomes in complete Freund adjuvant (14). Gonococcal ribosomal ribonucleic acid, ribosomal protein, cell walls, and pili required higher concentrations to obtain a positive skin test than did intact ribosomes. Kwapinski and Chong (18) used purified gonococcal antigens to elicit a CMI in rabbits. A nontoxic protein called B(-/+) was isolated from the cytoplasm of type 1 gonococci. B (-/+) was used to immunize rabbits prior to challenge with live gonococci in the anterior chamber of the eye. The corneas became cloudy within 24 to 48 h, and this was followed by a profuse discharge from the conjunctiva. By day 4 or 5 postinfection, the corneas were clear. In addition, the gonococci thrived when anti-B (-/+) antiserum was injected along with live gonococci into the anterior chamber of the eye.

HOST CELLULAR DEFENSES IN MENINGOCOCCAL INFECTION

Immunity to meningococcal infection is thought to depend primarily on the presence of bactericidal antibody. The production of this protective antibody follows natural infection (9) and vaccination with meningococcal polysaccharide antigens. Subjects who do not possess bactericidal antibody are at risk of developing meningococcal disease (6). Little attention has been paid to the possible protective role of CMI in this infection because of the evidence suggesting a dominant role for antibody in protection against meningococcal disease.

Meningococcal CMI Response

Greenwood et al. (10) studied the CMI response in patients with group A meningococcal meningitis and in normal subjects given group A meningococcal vaccine. They found that lymphocyte responsiveness to both phytohemagglutinin and meningococcal antigens was markedly depressed in patients with acute meningococcal infections. This defect was present whether lymphocytes were cultured in autologous or fetal calf serum. Patients also showed a transient increase in the degree of inhibition produced by group A meningococci in leukocyte migration assays. Meningococci of other groups produced a similar degree of inhibition. Vaccination with group A meningococcal polysaccharide vaccine had no effect on lymphocyte responsiveness to meningococcal antigens or on the inhibitory effect of group A meningococci on leukocyte migration. These negative results must be interpreted carefully, since patients with

acute meningococcal meningitis had positive leukocyte inhibition test results which were greater than those seen in recovered patients. Thus, there appears to be a difference in the level of responsiveness depending on the degree of antigenic stimulation.

ADCC and NK Cell Activity against Meningococci

Lowell et al. (20) reported that heat-inactivated serum samples from adults immunized with group C meningococcal polysaccharide vaccine, in cooperation with normal human peripheral blood mononuclear cells, could significantly decrease the viability of group C meningococci. Of the mononuclear-cell populations, K lymphocytes (null cells) and monocytes, but not T or B lymphocytes, were capable of effecting ADCC antimeningococcal activity in this system. The degree of killing in this system was dependent on the incubation time of the reactants, the concentration of the effector cells, and the amount of antiserum used in the assay. When specific antimeningococcal antibodies were absorbed from the serum, ADCC activity was abolished. ADCC antimeningococcal activity was also temperature dependent and could be abolished either by performing the assay at 4°C or by heating the effector cells to 46°C for 15 min prior to the start of the assay. Their data suggest that K cells may play a role in the host immune defense against certain bacterial pathogens. Smith and Lowell (33) reported that ADCC antimeningococcal activity was inhibited when human immune serum was preincubated with the polysaccharide of the immunizing vaccine. Furthermore, the activity against group A meningococci that was induced by heat-inactivated human immune serum was also inhibited by preincubation with that polysaccharide. Neither heterologous polysaccharide nor homologous protein or LPS meningococcal antigens inhibited the activity of either of these sera. Antibodies responsible for ADCC activity against the group C meningococci are also directed against the polysaccharide. The results of these inhibition experiments are, therefore, consistent with those of Roberts (27), who found that opsonophagocytosis of group C and A meningococci by neutrophils in cooperation with sera taken after immunization with group C and group A meningococcal polysaccharides, respectively, was totally inhibited by the homologous polysaccharide. Owing to natural exposure and carriage, most sera of adult humans contain antibodies to both capsular and subcapsular (protein and LPS) meningococcal antigens (9, 40). However, in contrast to the inhibitory action of the polysaccharide, homologous protein and LPS antigens were not inhibitory in this system. These data should not be interpreted as indicating that antibodies to protein and LPS antigens are incapable of inducing ADCC, since blocking the action of antibodies to protein or LPS would still leave antibodies to polysaccharide functionally available in the sera. The data do support the conclusion that if the antibodies to subcapsular LPS or protein are effective in ADCC, they must be of low titer, since blocking of antibodies to polysaccharide did not reveal antibacterial activity. This implies that, since ADCC activity occurs in the absence of added complement, this mechanism may be important to host immune defenses in areas where complement components are relatively low or nonfunctional, such as secretory surfaces including the nasopharynx and the respiratory, intestinal, and urogenital mucosa.

To compare the efficacies of the various immunoglobulin isotypes in ADCC against meningococci, Lowell et al. (22) used purified immunoglobulins from serum samples from

individuals immunized with group C polysaccharide and compared them with immunoglobulins purified from patients convalescing from disseminated meningococcal disease. They found that although IgA is nonbactericidal in the presence of complement, it can induce a cell-mediated antibacterial activity as effectively as IgG can. The amount of IgG required to induce cell-mediated antibacterial activity is similar to the amount required for complement-mediated killing. They further concluded that the amount of either postimmunization or convalescent-phase IgM required to induce complement-mediated killing is 16- to 20-fold smaller than the amount of IgG required. IgM is inferior to IgG in its ability to induce ADCC. In the cell-mediated system, post-immunization IgM is ineffective, and the amount of convalescent-phase IgM required for minimal activity is eight times the amount of convalescent-phase IgG required. Furthermore, the maximal antibacterial index induced by convalescent-phase IgM is 50% less than that which can be induced by IgG. These data suggest that IgG and IgA play a greater role than IgM in the ADCC directed against meningococci in host defense. Lowell et al. further reported (21) that IgA purified from serum samples of patients convalescing from disseminated group C meningococcal disease induced human monocyte-mediated ADCC in the absence of complement. The convalescent-phase IgA was directed specifically against the polysaccharide capsule, and the effective level for ADCC was less than 1 ng of polysaccharide antibody. ADCC activity again was dependent upon the length and the temperature of the test incubation and on the concentration of the monocytes.

CELLULAR RESPONSES IN ANIMALS IMMUNIZED WITH *N. MENINGITIDIS*

A number of aspects of the immune responsiveness of meningococcal infection are still elusive. CMI studies may provide a means of completing the assessment of these responses. There is a demonstrable cellular component to meningococcal immunity which, although antibody dependent, is mediated by cells and is complement independent. The few examples of specific CMI reactions to *N. meningitidis* are limited to migration inhibition in guinea pigs and humans (10, 26, 34, 35) and delayed-type hypersensitivity in guinea pigs (26). Pribnow et al. (26) sensitized guinea pigs to *N. meningitidis* group A by subcutaneous injection of viable meningococci. These animals were skin tested with heat-killed *N. meningitidis* cells, as cell wall preparation of meningococci, and a soluble somatic antigen prepared from the homologous organism. Control skin test substrates included heat-killed *N. gonorrhoeae* cells, purified protein derivative, and Hanks balanced salt solution. Positive 24-h skin reactions, characterized by induration that measured more than 25 mm², were produced only by heat-killed meningococci and with the cell wall preparations. The soluble somatic antigen produced only erythema. The meningococci also caused inhibition of migration of macrophages when peritoneal cells from the sensitized guinea pigs were used in the capillary tube MIF test. No inhibition of migration was produced with the control antigens. The delayed-type hypersensitivity reactivity was transferable with viable lymph node cells from the sensitized guinea pigs, but not with dead lymph node cells or with serum.

Sparkes (36) described a preparation of meningococcal antigens extracted in CaCl₂ which contained mostly outer membrane proteins and was strongly mitogenic for normal murine B lymphocytes. These meningococcal antigens

markedly impaired the in vivo T-cell responsiveness of murine splenocytes. Suppression of the normal splenic T cells occurred with both adherent and nonadherent splenocytes from meningococcal antigen-sensitized mice. B cells were much less affected by the suppression induced by the meningococcal antigens, and only adherent cells could convey in vitro the low-level impairment of B-cell proliferation. Strong T-cell suppression associated with a B-cell mitogen was also produced by *Mycobacterium bovis* BCG and *Corynebacterium parvum*. In another report (34), Sparkes showed that meningococcal antigens had adjuvant activity when administered to mice at the same time as a T-dependent antigen (sheep erythrocytes, [SE]), by increasing the splenocyte plaque forming response in a dose-related manner. However, when SE were given 1 day after meningococcal antigen injection, the subsequent plaque formation was diminished. This decrease was proportional to the dose of meningococcal antigen injected. Splenocytes taken from mice up to 5 days after meningococcal antigen injection actively inhibited plaque formation when mixed with splenocytes immunized with SE 4 days earlier. At 2 days after meningococcal antigen injection, the nonspecific inhibition of plaque formation was due mainly to adherent spleen cells, whereas at 5 days, nonadherent cells had acquired the inhibitory activity. It appears that the degree of activation of adherent cells by meningococcal antigen modulates the subsequent development and secretion of anti-SE antibody-forming cells.

Micusan et al. (24) used an extract from group Y meningococci known to contain protein antigens common to other meningococci to determine the immune response in mice to meningococci. Using delayed-type hypersensitivity as a measure of cell-mediated responsiveness, they could not detect any CMI. Melancon-Kaplan et al. (23), using spleen cells from mice infected with meningococci, demonstrated depressed in vitro plaque-forming cells responses to T-dependent (SE) and T-independent (trinitrophenol [TNP] LPS and TNP-Ficoll) antigens. The inhibition was observed over a wide range of antigen concentrations. The decreased responsiveness of splenocytes from infected mice was due to a selective impairment of B-cell function. Helper-T-cell activity was intact in infected mice, as shown by the ability of T-enriched lymphocytes to cooperate with normal B-enriched lymphocytes in the generation of an anti-SE response. Accessory macrophage function was preserved, since adherent spleen cells from mice inoculated with bacteria were shown to produce normal or increased levels of interleukin-1 and were able to cooperate with normal non-adherent spleen cells in the generation of plaque-forming cell responses against SE. Addition of peritoneal cells from normal animals or extraneous interleukin-1 both failed to restore normal plaque-forming cell responses in cultures of splenocytes from infected mice. B-enriched lymphocytes from infected mice produced poor anti-SE responses when cultured with either concanavalin A supernatant or T-enriched lymphocytes from normal or infected mice. Therefore, the immunological unresponsiveness associated with a meningococcal infection was attributed to a meningococcus-induced defect(s) in B-cell function. In vivo polyclonal B-cell activation leading to clonal exhaustion does not play a major role in the depression of humoral responses, since meningococcal infection induces little or no polyclonal immunoglobulin secretion.

CONCLUSIONS

During the last several decades, there have been sporadic reports concerning the presence of CMI in response to infection with *Neisseria* spp. This includes reports both on the classical manifestations of CMI, such as delayed-type hypersensitivity skin testing, in vitro lymphocyte transformation, and production of soluble lymphokines, as well as more contemporary studies on the activity of cells involved in ADCC and NK cell activity. These reports have been overshadowed by the large number of studies suggesting that immunity to these bacteria is antibody mediated through either complement activation or opsonization. There are several observations which argue against the simplistic notion that only humoral immunity is involved in protecting an individual against infection. These are as follows. (i) Most of the studies of the role of antibody in these infections have been done with immune serum. However, both gonococci and meningococci produce local infections, where serum antibody may not be present. (ii) Complement is deficient or present in only low concentrations in areas where these two species initiate their infections. Furthermore, the predominant immunoglobulin present in the genitourinary tract (for gonococci) and the nasopharynx (for meningococci) is of an isotype (IgA) that does not fix complement. (iii) Antibodies specific for gonococci can be isolated from serum samples of individuals with histories of multiple uncomplicated gonococcal infections. The fact that these individuals develop multiple infections while this antibody is present argues against the postulate that this antibody is protective. Early work on CMI in these infections demonstrated no consistent correlation between the development of specifically sensitized cells and protection. More recent investigations of the ability of human lymphocytes to participate in NK-cell and ADCC activities against gonococci and meningococci suggest that we need to reevaluate the conclusions on the identity of protective immune mechanisms to these two bacteria. This is particularly important as efforts are undertaken to develop new, more effective vaccines for the diseases caused by these organisms.

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