Cellular Basis of Decreased Immune Responses to Pneumococcal Vaccines in Aged Mice

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Previously, model systems were developed in our laboratory to study murine immune responses to the 23-valent pneumococcal polysaccharide vaccine Pnu-Imune, both in vivo and in vitro (M. Garg and B. Subbarao, Infect. Immun. 60:2329–2336, 1992; M. Garg, A. M. Kaplan, and S. Bondada, J. Immunol. 152: 1589–1596, 1994). Using these systems, we found that aged mice did not respond to the vaccine in vivo or in vitro. Cell separation studies showed that the unresponsiveness of the aged spleen cells to the vaccine was not due to an intrinsic B-cell defect or to T-cell-mediated immunosuppression but resulted from an accessory cell deficiency. Irradiated spleen cells from young mice enabled the old mouse spleen cells to respond to the vaccine. Interestingly, irradiated spleen cells from old mice also restored the vaccine responsiveness in old mice but were required in greater numbers than the young mouse spleen cells to induce similar levels of response. The accessory cell was an adherent cell that could be removed by passage through Sephadex G-10 and thus may be a macrophage. Accessory function could also be provided by the cytokine interleukin-1 (IL-1), IL-4, or IL-5 but not IL-2 or IL-6. Thus, one reason for the deficient immune response to pneumococcal vaccine in aged mice is a quantitative defect in adherent accessory cells.

Immune responses are decreased in the aged, resulting in an increased incidence of infections in the elderly (43, 45, 58). The thymus atrophies with age, and T cells exhibit a number of abnormalities such as reduced interleukin-2 (IL-2) secretion but an increased production of IL-6 and gamma interferon (IFN-γ) (12, 14, 19, 32, 41). Although a decline in T-cell function is the major cause of reduced immune function in the aged, B cells and macrophages also function less effectively in the elderly (21, 26, 46, 56). Thus, B cells from aged mice proliferated poorly in response to stimulation with activated T cells (36, 53) or with anti-μ plus IL-4 (55) and were less susceptible to tolerance induction (59). Also, fewer B cells were generated from bone marrow precursors in the aged, but neither the B-cell repertoire nor their life span was altered substantially (27, 57, 60, 61).

One of the major consequences of the senescent immune system is an increased incidence of pneumococcal pneumonia, which is a leading cause of death in the elderly (2, 28). Moreover, there has been an increase in the occurrence of antibiotic-resistant pneumococcal bacteria (37). Serum antibodies to the capsular polysaccharides (PS) of the pneumococcal bacteria provide protection against the pneumococci, but such antibodies are not present at sufficient levels in the aged (2, 20, 28). A vaccine, made up of purified capsular PS from 23 commonly occurring serotypes of pneumococcal bacteria, was developed to increase the levels of circulating antibodies to pneumococcal bacteria in older people (10). The efficacy of this vaccine in the elderly has been extremely variable (9, 47, 49), and it was ineffective in small children (5) as well as in patients with AIDS (48). However, the precise mechanisms underlying the poor immunogenicity of the vaccine in these populations have thus far not been elucidated.

Recently we developed a murine model with which to study the cellular basis of immune response to the pneumococcal PS vaccine (17, 18), using the whole vaccine as immunogen and vaccine-coupled sheep erythrocytes (SRBC) to detect antibody-secreting cells (18). This system measured antibody responses to 21 of 23 PS in the vaccine, and the responses measured with the whole vaccine were 50% as efficient as with the individual PS (18). The murine model system also exhibited an age-associated decline in the immune response to the vaccine, which was consistent with the previously reported decreased responsiveness of the aged to type III pneumococcal PS (51). The responses of the old mice to the vaccine were substantially enhanced by the adjuvant, monophosphoryl lipid A (MPL), and the steroid hormone dihydroxy epiandrosterone (DHEA), agents that have been shown to respectively stimulate accessory function and alter cytokine secretion patterns from T cells (1, 14, 16, 18, 42). Also, our studies showed that spleen and mesenteric lymph nodes but not peripheral lymph nodes responded to the vaccine, which is consistent with the well-known splenic dependence of the antibody response to pneumococcal PS (2, 18).

Using a recently established in vitro culture system to reproducibly obtain vaccine-specific immune responses, we showed that the vaccine induced a good response without T cells but was regulated by T-cell-derived lymphokines (17). Thus, IL-5 enhanced the vaccine response whereas IFN-γ inhibited the vaccine response. Further, the in vitro system replicated the in vivo findings concerning the differential ability of spleen and lymph node B cells to respond to the vaccine. The unresponsiveness of the lymph node cells to the vaccine was due to a deficiency in accessory cell function which could be reconstituted by splenic adherent cells (17). Many characteristics of the vaccine response are similar to those of trinitrophenyl (TNP)–aminoethyl-carboxymethyl-Ficoll (Ficoll), a prototype of type 2 T-independent (TI) antigens, but unlike TNP-Ficoll, acute depletion of T cells did not diminish the in vitro vaccine response.

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Because of this and other differences such as the susceptibility to regulatory T cells, we have classified TNP-Ficoll as a TI-2A antigen and vaccine and other pneumococcal PS as TI-2B antigens (6).

Although the phenomenon of age-associated decline in antibody response to the pneumococcal PS in humans as well as mice has been known for over 25 years, the cellular basis of this defect has not been precisely defined because of a lack of good in vitro systems. In this study, we used our recently developed in vitro model system to investigate the cellular basis of the decreased vaccine response in the aged. We found that the hyporesponsiveness of the aged to the vaccine was due not to an increase in T-cell-mediated suppressive influences but to a lack of accessory cell function. The response of the aged mouse spleen cells was restored by supplementation with accessory cells from young mice or a variety of cytokines such as IL-1, IL-4, and IL-5.

MATERIALS AND METHODS

Mice. Young (14- to 22-week-old) and old (20- to 26-month-old) BALB/c mice as well as mice of intermediate ages were obtained from the National Institute of Aging through Charles River Laboratories and Sprague Daley. Old mice that had tumors or splenomegaly or were weak or sick were excluded from all analyses. About 10 to 20% of the old mice had such characteristics, which required their elimination from the study, whereas none of the young mice exhibited such properties.

Reagents. Pnu-Immune 23, a 23-valent pneumococcal vaccine, was obtained from Lederle Laboratories, Pearl River, N.Y. TNP-*Brucella abortus* was prepared as described before (25). TNP-Ficoll was a kind gift from John Inman, National Institutes of Health, Bethesda, Md. Recombinant-derived human IL-1 and murine IL-2 were obtained from Genzyme Corp. (Boston, Mass.). Recombinant-derived murine IL-4 and murine IL-5 were obtained as supernatants from HeLa cells transfected with specific genes, and the transfected cell lines were obtained from T. Honjo, Kyoto University, and Kim Bottomly, Yale University. In some experiments, purified IL-4 and IL-5 (Genzyme) were used. Supernatant from a turkey gamma globulin-specific Th2 type of helper cell line (TGG Sup) was obtained from Don Cohen, University of Kentucky. It contained IL-4 and IL-5 but no detectable IL-2 activity, and the lymphokines were partially purified by ammonium sulfate precipitation (11). Purified recombinant murine IL-6 was a kind gift of the Genetics Institute (Boston, Mass.).

Preparation of cells. Spleen cells from unimmunized mice were obtained by teasing the spleens by compression with a syringe plunger on a petri dish in Hanks balanced salt solution. The spleen cells were allowed to settle for 2 min to settle any debris, and the supernatant was centrifuged at $400 \times g$ to obtain lymphocytes. The lymphocytes were washed twice with Hanks balanced salt solution before use and were suspended in tissue culture medium described below. The tissue culture medium, shown to support in vitro responses from B cells, consisted of a 1:1 mixture of Iscove's modified Dulbecco's medium and Ham's F12 medium supplemented with L-glutamine, 2-mercaptoethanol, insulin, transferrin, progesterone, trace elements, and gentamicin as described by Mosier (35) and Subbarao and Mosier (52) (IF12 medium) plus 10% fetal calf serum (FCS; HyClone Laboratories, Logan, Utah).

Spleen cells were depleted of adherent accessory cells by adhering to plastic for 1 h and passage of the nonadherent cells through Sephadex G-10 columns as described by Ly and Mishell (30). T cells were removed by treating spleen cells with monoclonal antibodies (MAbs) to the Thy 1.2, CD4, and CD8 antigens plus agarose-adsorbed rabbit complement (PelFreeze Laboratories) once or twice as described by us previously (18, 25). The proliferative response of the T-cell-depleted spleen cells to the T-cell mitogen concanavalin A was decreased by 95 to 99%. Spleen cells before and after depletion of T or adherent cells were irradiated at 3,000 rads with γ rays from a ^{137}Cs source whenever they were used as a source of accessory cells. Such irradiated cells were tested routinely for a vaccine response by culturing them with an optimal dose of the vaccine and were found to be completely unresponsive.

Adherent accessory cells were obtained by the procedure described by us previously (17). Spleen cells, suspended in IF12–1% FCS, were incubated in a tissue culture flask in a 5% CO_2 atmosphere at 37°C for 1 h, and the nonadherent cells were removed by aspiration. The remaining cells were incubated for another 30 min, and the nonadherent cells were removed. Fresh medium was added to the flask, and incubation was continued overnight to remove dendritic cells, which become nonadherent during this period. The adherent cells were collected from the flask by treatment with 2 mM EDTA in phosphate-buffered saline (PBS) for 5 min. The cells were washed twice with PBS and were used for flow cytometry or as accessory cells.

Flow cytometric measurement of phagocytic cells. The spleen cells or accessory cells (10^6/ml) were resuspended in RPMI–1% FCS and exposed to fluorescent latex bead suspension (Polyscience Inc., Warrington, Pa.) for 30 min at 37°C
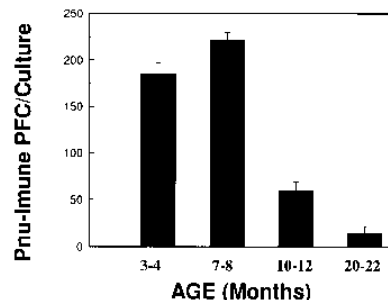

FIG. 1. Effect of age on the immune response to vaccine. Spleen cells (2×10^6) from BALB/c mice of various age groups were cultured in vitro as described in Materials and Methods with 0.0115 μg of Pnu-Immune vaccine for 5 days, and the vaccine-specific antibody-forming cells were measured by using Pnu-Immune coupled SRBC. Two to three mice were used for each age group, and the results from one of two similar experiments are shown. The pattern of age-dependent changes in the response remained the same when 0.0015 or 0.115 μg of vaccine was used as the immunogen. The values represent the arithmetic means of responses of triplicate cultures, and the bars represent SE values.

in a water bath. The cells were separated from free beads by being washed twice with cold 2 mM EDTA–PBS buffer and were analyzed on a FACScan flow cytometer (Becton Dickinson, Mountain View, Calif.). The cells that took up the latex beads appear as sharp peaks with very high fluorescence intensity. The number of Mac-1-positive cells was determined by staining with rat anti-mouse Mac-1 MAb plus fluorescein isothiocyanate-conjugated goat anti-rat immunoglobulin G (Southern Biotechnology Associates, Birmingham, Ala.).

In vitro culture conditions. The culture conditions were the same as described before (17). Briefly, 2 million spleen cells from young or old mice were cultured in triplicate with or without the indicated doses of antigen in 1.0 ml of IF12–5% FCS in 24-well Costar (Cambridge, Mass.) dishes for 5 days, or as indicated, at 37°C in a humidified atmosphere with 5% CO_2.

PFC assay. The cultures were assayed for antigen-specific antibody-secreting cells by a slide modification of the Jerne hemolytic plaque-forming cell (PFC) assay, using TNP or vaccine-coupled SRBC as described by us earlier (18, 25). Briefly, 0.5 ml of packed SRBC was coupled with 1.0 mg of the 23-valent Pnu-Immune vaccine, using 0.1% chromium chloride, by rotating the tube gently for 10 min at room temperature (18). The vaccine-coupled SRBC were washed three times with normal saline and were used on the same day. The clarity of the plaques was enhanced by adding 0.6% polyethylene glycol 8000 to the plaquing mixture. Previously we demonstrated that these vaccine-coupled SRBC were ~50% as efficient as SRBC coupled with individual capsular pneumococcal PS in detecting the PFC response to any particular component of the vaccine. Also, we showed that both the in vitro and in vivo PFC responses were mainly against the capsular PS and that the response to the contaminating cell wall PS was estimated to be 10 to 14% of the response measured by the vaccine-coupled SRBC. TNP-SRBC were prepared by reacting SRBC with 2,4,6-trinitrobenzene sulfonic acid at room temperature, and any unreacted reagent was removed by treating washed TNP-SRBC with glycylglycine.

The cultures were assayed for PFC by using both vaccine-coupled SRBC and unconjugated SRBC. Any response measured with unconjugated SRBC was subtracted from the values obtained with vaccine-coupled erythrocytes. Results are represented as means ± standard errors (SE) of triplicate samples.

RESULTS

Unresponsiveness of spleen cells from old mice to Pnu-Imune vaccine in vitro. Our earlier studies showed that neither spleen nor lymph node cells from old mice responded to an in vivo challenge with the 23-valent Pnu-Imune vaccine (18). To understand the cellular basis of this unresponsiveness, we studied these responses in vitro, using a culture system described by us recently (17). When spleen cells from young and old mice were cultured in vitro with an optimal dose of the vaccine, spleen cells from young but not old mice generated vaccine-specific PFC (Fig. 1). In comparison with the young mice, the vaccine response declined by about 70% by 10 to 12 months of age, and at 20 to 22 months of age, only 10% of the response remained. In agreement with this finding, our previous in vivo studies showed that 22-month-old mice did not respond well to the vaccine (18). To be certain that this de-
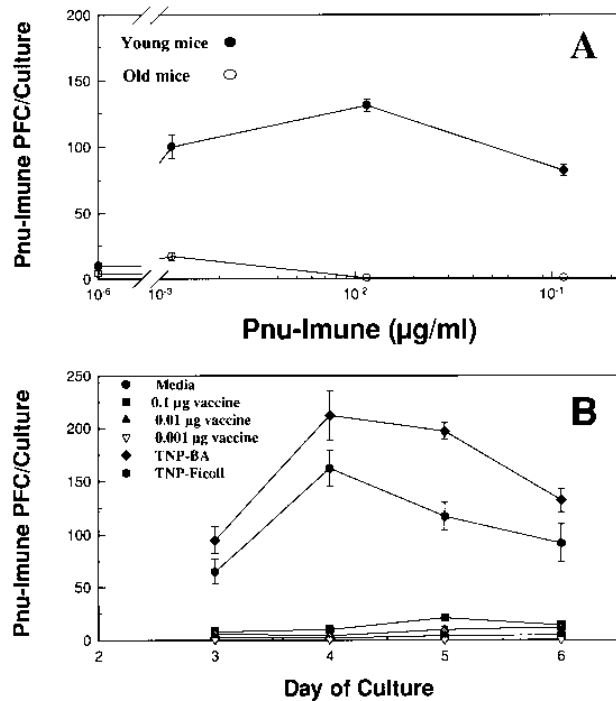
FIG. 2. Kinetics of in vitro immune response to the vaccine. (A) Spleen cells from young (3 to 5 month-old) and old (24-month-old) BALB/c mice were cultured for 5 days with various doses of the vaccine. (B) Spleen cells from old mice (22 to 24 months) were cultured with various doses of vaccine, TNP-BA, or TNP-Ficoll for the indicated periods of time. The vaccine-specific PFC responses are measured as described in Materials and Methods. The values represent arithmetic means ± SE of triplicate cultures. Results from one of three similar experiments are shown.
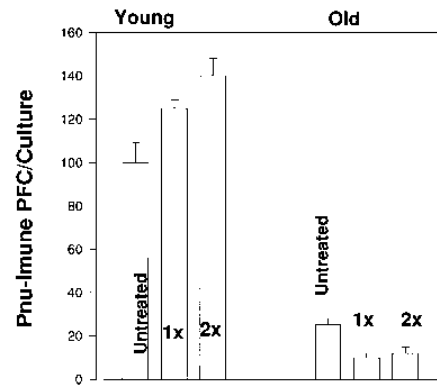

FIG. 3. Role of T cells in the vaccine response. T cells were removed by treating young or old mouse-derived spleen cells with a cocktail of anti-T-cell antibodies and complement either once or twice (bars with 1x and 2x) as described in Materials and Methods. The T-cell-depleted spleen cells were cultured with 0.0115 μg of the vaccine for 5 days, at which time the number of vaccine-specific PFC was measured as described above. This experiment was repeated at least three times with similar results. In all experiments, the treated and untreated spleen cells were also cultured with a 0.00115-μg dose of the vaccine with a similar outcome. When corrected for B-cell numbers, the differences between the responses of untreated and T-cell-depleted (1x or 2x) young spleen cells were not statistically significant ($P > 0.07$). For old mouse spleen cells, the responses were very small but the differences were statistically significant ($P = 0.007$ and 0.02, respectively, for once- and twice-T-cell-depleted cells).

crease in vaccine response was not due to a change in the dose response or in kinetics, spleen cells from old mice were cultured with various doses of the vaccine for various periods of time. As shown in Fig. 2A, spleen cells from old mice did not respond to any dose of the vaccine whereas the young mouse-derived cells responded well. Furthermore, old mouse spleen cells did not respond to the vaccine at any tested time after initiation of culture (Fig. 2B). The spleen cells from old mice responded well to other TI antigens such as TNP-*Brucella abortus* and TNP-Ficoll (Fig. 2B).

Absence of vaccine response in old mice was not due to T-cell-mediated suppression. Although the PFC response to pneumococcal PS is T-cell independent (3, 18), several investigators have shown the PS response to be regulated by suppressor T cells (3, 4, 7, 8, 54). To determine if the vaccine unresponsiveness in old mice was due to such T-cell-mediated suppressive influences, T cells from young and old mice were depleted by treatment with anti-T-cell-specific MAbs and complement once or twice. As shown in Fig. 3, elimination of T cells by single or double treatment did not reduce the PFC response from young mice spleen cells, in agreement with the T-cell-independent nature of this response as reported by us earlier (18). Moreover, the T-cell depletion failed to restore responsiveness to the vaccine in spleen cells from old mice. The T-cell-depleted spleen cells from young and old mice responded to TNP-BA as well as the untreated spleen cells (data not shown). As described in Materials and Methods, the T-cell depletion protocol reduced the proliferative response to the T-cell mitogen concanavalin A by 95 to 99%. Also, in young and aged mice, this treatment reduced the PFC re-

sponse to TNP-Ficoll, consistent with the previously reported T-cell-dependent nature of this TI-2A antigen (15, 17, 29, 33).

Splenic accessory cells from young mice restore the vaccine response of old mice. It is conceivable that old mouse spleen cells are deficient in a cell type that is necessary for the vaccine response and is present in the young mice in adequate numbers. Therefore, various numbers of irradiated young mouse spleen cells were added to the cultures of spleen cells from old mice plus vaccine. As shown in Fig. 4, irradiated young mouse spleen cells helped the old mouse spleen cultures to respond to the vaccine very well, suggesting that old mouse spleen is deficient in a splenic accessory cell. Interestingly, addition of
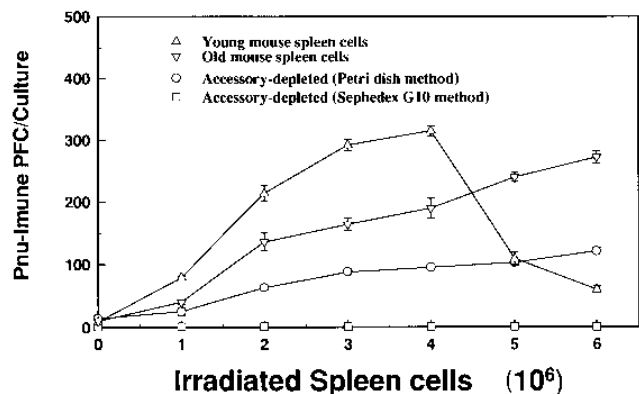

FIG. 4. Splenic accessory cells from young or old mice restore the antibody response to Pnu-Imune vaccine. Spleen cells from old mice were cultured with 0.0115 μg of the vaccine with various numbers of irradiated spleen cells from young (4 to 5 month-old) or old (20- to 24-month-old) mice for 5 days. The adherent cells were removed from the young mouse spleen either by incubation on plastic petri dishes or by passage through Sephadex G-10 columns. The mean values of vaccine-specific PFC responses from triplicate cultures are shown along with the SE bars. Similar results were obtained with all doses of the vaccine tested, but the results with 0.0115 μg of the vaccine are shown. Representative results from an experiment that was repeated three times are shown.

TABLE 1. Purity of adherent cells assessed by their phagocytic capacity[a]

Cells	% Mac-1 positive	% Cells containing latex beads
Spleen	6	9.5
Nonadherent	1.2	5.3
Adherent	40.7	54.5

[a] Various cell populations (10^6), prepared as described in Materials and Methods, were incubated with 1.0 µl of latex beads or stained with anti-Mac-1 MAb. The cells were washed as described in Materials and Methods and then analyzed on a FACScan flow cytometer. Representative results from one of three such experiments are shown.

irradiated old mouse spleen cells also restored the vaccine responsiveness of unirradiated cells from old mice (Fig. 4). Control experiments showed that the irradiated young or old mouse spleen cells did not respond by themselves to the vaccine. Larger numbers of the accessory cells from old mice were needed to induce as good a response as for the young mouse-derived irradiated spleen cells. Thus, the deficiency appeared to be a quantitative one in old mice. Removal of T cells from the irradiated spleen cells did not affect their ability to reconstitute the vaccine response of old mice in these in vitro cultures (data not shown).

To determine if macrophage-like adherent cells are necessary to detect accessory function in the spleen cells, adherent cells were depleted from young mouse spleen cells by adherence on plastic or by passage through a Sephadex G-10 column (Fig. 4). Results presented in Fig. 4 demonstrated that the critical cell necessary for reconstitution of the vaccine response was an adherent cell. Notably, these adherent cells were removed more efficiently by Sephadex G-10 column than by adherence to plastic (Fig. 4). Similarly, passage of old mouse spleen cells through the Sephadex G-10 column reduced their ability to reconstitute the vaccine response of the old mice (data not shown). To further characterize the accessory cells, they were purified by overnight adherence to plastic and the number of phagocytic cells was determined by their ability to take up fluorescent latex beads, using a flow cytometer. As shown in Table 1, the adherent cells were enriched for cells that take up the latex beads. Also, the adherent cells were enriched for cells that express the macrophage-specific Mac-1 antigen (Table 1). The numbers of Mac-1-positive cells was slightly less than the number of cells that took up latex beads, which may be because of the reported loss of Mac-1 from some activated macrophages (31). In accordance with this finding, up to 15% of the Mac-1-negative cells expressed F4/80, another macrophage-specific marker. However, most of the remaining (~40%) adherent cells were found to be B cells, which should not contribute to the vaccine response since they were always irradiated. In every experiment, we verified that the irradiated splenic accessory cells (from young or old mice) did not respond to the vaccine on their own but restored the vaccine response of the spleen cells from aged mice.

Cytokines can substitute for accessory cell function. The function of splenic macrophages can be provided by IL-1 in a variety of TI-antigen responses, including the PFC response to the vaccine (6, 17, 24, 25, 40, 50). Also, macrophages from old mice have been shown to produce less IL-1 than their young counterparts (26). Therefore, spleen cells from old mice were cultured with vaccine in the presence of IL-1, which also permitted these cells to respond to the vaccine extremely well (Fig. 5A). Notably, a T-cell line-derived supernatant (TGG Sup), which contained IL-4, IL-6, and IL-5 but not IL-2, supported a vaccine response from spleen cells from old mice.
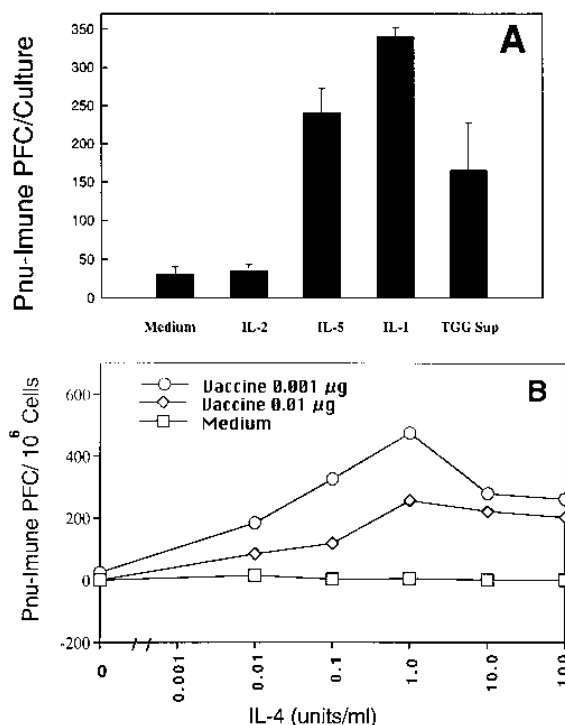

FIG. 5. Effects of lymphokines on the PFC response of aged mouse spleen cells to the vaccine. Spleen cells from old mice were cultured with 0.0115 µg of the vaccine in the presence of an optimal dose of each cytokine and the vaccine. (A) TGG Sup- and HeLa cell-derived recombinant IL-5 were used at 1:100 dilution of the stock. Recombinant IL-1 and IL-2 were used at 10 and 50 U/ml, respectively. The mean values of vaccine-specific PFC responses from triplicate cultures are shown along with the SE values. (B) Recombinant IL-4 was used at the indicated concentrations. All other conditions were the same as for panel A. Results from representative experiments that were repeated four (A) and two (B) times are shown.

When tested individually, IL-5 (Fig. 5A) and IL-4 (Fig. 5B) were very effective in restoring the vaccine response from aged mice. Neither IL-2 (a cytokine that is deficient in the aged) nor IL-6 (a cytokine that is elevated in old mice) induced a vaccine response in old mouse spleen cells. Importantly, when tested with young mice, both IL-2 and IL-6 did not inhibit the vaccine response at all doses tested (1 to 100 U/ml). Thus, changes in these T-cell-derived cytokines with age are not likely to be the primary cause of the decreased vaccine response in the old mice.

Next we tested if cytokines can act on B cells directly and induce a vaccine-specific response. B cells were purified by removing macrophages by a combination of plastic adherence and passage through Sephadex G-10 column, while the T cells were eliminated by treatment with three anti-T-cell antibodies plus complement as described in Materials and Methods. As shown in Fig. 6, the highly purified B cells responded poorly to the vaccine even in the presence of IL-5. Although, the magnitude of response in the presence of IL-1 alone was higher, it was still less than that obtained in the presence of both cytokines. The PFC responses in the presence of the individual cytokines (IL-1 and IL-5) were found to be variable, whereas the response in the presence of both cytokines was always substantially high in six different experiments.

DISCUSSION

These studies have shown that the in vivo unresponsiveness of aged mice to Pnu-Imune vaccine can be reproduced in vitro.
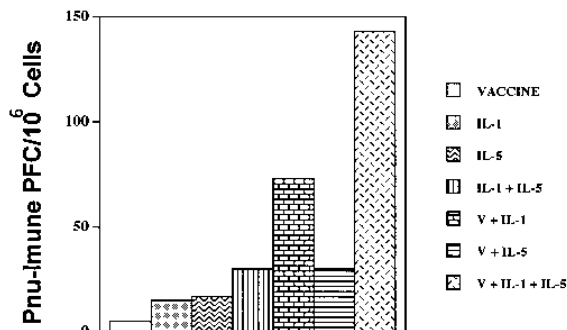
FIG. 6. IL-1 and IL-5 can induce a vaccine-specific PFC response from purified B cells from old mice. Adherent and T-cell-depleted B cells were purified as described in Materials and Methods. Recombinant IL-1 and IL-5 were used at 25 and 10 U/ml, respectively. All other experimental conditions are the same as for Fig. 5. Results are representative of three experiments with similar results. The SE bars are too small to be seen.

Cell separation analysis demonstrated that the lack of response was not due to T-cell-mediated suppressive influences but was primarily due to a deficiency in a splenic adherent accessory cell. Also, these data suggested that B cells in the aged mice did not have an intrinsic defect in their ability to respond to the vaccine. These observations are consistent with two earlier in vivo observations. First, Smith found that the numbers of PS-binding B cells were not reduced in aged mice in spite of a low antibody response (51). Second, MPL, an agent that has been shown to activate macrophages, restored vaccine responsiveness in old mice (18). In vitro, young and aged mice responded similarly to TNP-BA, a TI-1 antigen which has mitogenic and macrophage activating properties which may overcome such defects in aged mouse B-cell responses (6). We found that the effect of age on in vitro responses to TNP-Ficoll was variable, with only a 20 to 30% decrease (data not shown). Previous studies found that the in vivo responses to TNP-Ficoll are reduced in the aged, presumably as a result of regulation by anti-idiotypes, which may not be made efficiently in short-term in vitro cultures, explaining a lack of age-associated defect in vitro (21).

Baker et al. also found that MPL enhanced the immune response to type III pneumococcal PS, presumably as a result of its ability to inactivate a suppressor T cell (4), which can explain its adjuvanticity for the vaccine response in the aged mice (18). As shown here, T-cell-mediated suppression was not the only cause of a poor response of aged mice to pneumococcal PS, since T-cell depletion failed to induce a vaccine response from aged mouse B cells.

Interestingly, the accessory cell defect was only quantitative, since irradiated spleen cells from old mice were also effective in eliciting a vaccine response from old mice but a larger number of such old mouse accessory cells than of the young mouse-derived spleen cells was needed. The ability of IL-1, an accessory cell-derived cytokine, to replace the need for these additional accessory cells suggests that the accessory cells from aged mice may be deficient in IL-1 secretion, and such a deficiency has been reported (26). This IL-1-correctable accessory cell defect for the vaccine response was similar to an antigen-presenting cell defect, whereby splenic macrophages were specifically unable to stimulate a syngeneic mixed lymphocyte reaction in aged mice unless exogenous IL-1 was added (46). Direct measurements of IL-1 secretion are needed to determine if the accessory cells from aged mice produce less of the cytokine per cell or if there is a reduction in the total

number of cells that produce normal levels of the cytokine. Previous studies concerning a decline in IL-1 production in aged mice did not distinguish between these two possibilities.

The ability of IL-5 to augment the vaccine response in the aged mice was surprising because the T-cell production of the lymphokines IL-4, IL-5, and IL-6, characteristically made by Th2 cells, was reported to be elevated in old mice (12, 14). Notably, Daynes and Araneo reported that IFN-γ, a Th1-derived lymphokine, was also elevated, while production of another Th1-dependent lymphokine, IL-2, was severely reduced in the aged mice in comparison with young mice (12). As shown by us earlier and in this study, the vaccine response was very low in aged mice both in vitro and in vivo. The low response to the vaccine in vivo may be due to the increase in the levels of IFN-γ, which we had previously shown to be inhibitory to the vaccine response (17). Hence, the vaccine response may be low in spite of elevated levels of IL-5, as a result of the inhibitory effects of IFN-γ in vivo. The in vitro cultures showed that in addition to such possible inhibitory effects by Th1-derived cytokines, an accessory cell deficiency also existed in old mice, since addition of splenic adherent cells but not removal of T cells by complement-mediated lysis restored responsiveness. However, we cannot rule out the possibility that non-T cells such as natural killer cells produce IFN-γ in vitro in T-cell-depleted cells. We are attempting to address this issue by using specific MAbs to block the effects of IFN-γ and by depleting natural killer cells.

Daynes and colleagues have postulated that the phenomenon of immune senescence may be related to an age-associated decrease in the production of the hormone DHEA, since it appears to modulate the patterns of cytokine secretion by T cells, leading to a decrease in IL-2 and increase an in IL-4, IL-5, IL-6, and IFN-γ (12–14). These workers successfully altered the pattern of cytokine secretion by T cells from aged mice as well as the immune responses to T-cell-dependent antigens following acute or chronic therapy with DHEA (12, 14). Our ability to reconstitute the in vivo vaccine response by acute treatment with DHEA is also consistent with a DHEA-induced decrease in IFN-γ (16). However, the accessory cell deficiency demonstrated here raises the question of whether the primary effect of DHEA is on T cells or accessory cells. Recently we have been able to reproduce the effect of DHEA by restoring the vaccine response from old mouse spleen cells in vitro (our unpublished observations), and therefore the site of action of the hormone can now be determined in vitro.

It is interesting that the spleen cells from aged mice resembled peripheral lymph node cells from young mice, which also did not make an antibody response to the vaccine or to all TI-2 antigens studied, in vivo as well as in vitro (6, 17, 18, 22–25). In both situations, elimination of T cells did not restore the vaccine response, suggesting that T-cell-mediated suppression was not the primary cause of unresponsiveness (6, 17, 23, 25). Furthermore, in both cases, splenic accessory cells reconstituted the response and the accessory cell defect was a quantitative one. In the young mouse-derived lymph node as well as in the old mouse spleen, both IL-1 and IL-5 substituted very well for the splenic accessory cells in eliciting a vaccine response (6, 17). Lymph nodes do not appear to develop the accessory function to adequate levels at any time in the life of the mouse, since both young and old mice cannot respond to a subcutaneous challenge with the vaccine (18). The spleen attains peak levels of such accessory cells by 3 to 4 months of age, when the vaccine responses are maximum, and their function begins to decline after about 10 to 12 months of age, resulting in a progressive loss of the vaccine response.

Our data suggest that IL-1 and IL-5 act directly on B cells

from aged mice but not by stimulating T cells or some other accessory cells to produce cytokines that can augment the B-cell response. However, we cannot rule out the possibility that they inhibit the production of the inhibitory cytokine IFN-γ by non-T cells such as natural killer cells. Studies by Pike and Nossal (40) and by Sinha et al. (50), with TNP-Ficoll-responsive B cells in single-cell cultures, showed that IL-1 can act directly on TI-2A antigen-responsive B cells. Also, our studies showed that TNP-Ficoll can induce a proliferative signal in lymph node B cells but not differentiation, a step facilitated by the addition of IL-1 or IL-5 (6, 24). Since the vaccine behaves like a TI-2 antigen, it is not surprising that the cytokines act directly on B cells to induce a vaccine-specific PFC response. Currently we are determining the role of IFN-γ in the vaccine response, using specific MAbs.

The accessory cell deficiency demonstrated here suggests that the efficacy of the vaccine may be increased for the elderly population by exploring adjuvants that can increase IL-1 production in vivo. Agents that can counteract the inhibitory effects of IFN-γ may also be useful in augmenting vaccine efficacy in the elderly. In accordance with this observation, preliminary studies showed that the vaccine responses of the aged mice can be enhanced by supplementation with IL-10, a cytokine that can antagonize IFN-γ production (29a, 34). Also, strategies to make the vaccine T-cell dependent may not be as important for older individuals (6, 38, 44), since the necessary T-cell-derived cytokine, IL-5, is already high in aged mice but is unable to help the vaccine response. In summary, these studies showed for the first time that the hyporesponsiveness of older animals to pneumococcal PS vaccine is due to an accessory cell deficiency and have suggested strategies to increase the vaccine efficacy for this population.

ACKNOWLEDGMENTS

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Editor: V. A. Fischetti