![UKnowledge logo](University of Kentucky UKnowledge)

University of Kentucky
UKnowledge

Microbiology, Immunology, and Molecular
Genetics Faculty Publications

Microbiology, Immunology, and Molecular
Genetics

6-1992

Immune Responses of Systemic and Mucosal Lymphoid Organs to Pnu-Imune Vaccine as a Function of Age and the Efficacy of Monophosphoryl Lipid A as an Adjuvant

Manju Garg
University of Kentucky

Subbarao Bondada
University of Kentucky, bondada@uky.edu

Right click to open a feedback form in a new tab to let us know how this document benefits you.

Follow this and additional works at: https://uknowledge.uky.edu/microbio_facpub

Part of the Immunology and Infectious Disease Commons, Medical Immunology Commons, Medical Microbiology Commons, and the Molecular Genetics Commons

Immune Responses of Systemic and Mucosal Lymphoid Organs to Pnu-Imune Vaccine as a Function of Age and the Efficacy of Monophosphoryl Lipid A as an Adjuvant

Immune Responses of Systemic and Mucosal Lymphoid Organs to Pnu-Imune Vaccine as a Function of Age and the Efficacy of Monophosphoryl Lipid A as an Adjuvant

MANJU GARG AND BONDADA SUBBARAO*

Department of Microbiology and Immunology, Sanders-Brown Center on Aging, University of Kentucky, Lexington, Kentucky 40536-0230

A murine model system was established to study immune responses to the Pnu-Imune vaccine, which is made up of 23 different pneumococcal capsular polysaccharides. In this animal model, antibody-forming cell responses to 21 of 23 individual polysaccharides in the vaccine were detected. The Pnu-Imune vaccine elicited good antibody responses from the spleens and mesenteric lymph nodes (MLN) of young mice, whereas a variety of other peripheral lymph nodes were unresponsive. The immunoglobulin M plaque-forming cell (PFC) response in the spleen to the Pnu-Imune vaccine (given intraperitoneally or subcutaneously) decreased dramatically with increasing age. However, the spleen and MLN differed in their susceptibility to an age-associated decline in immune function. While the PFC responses in the spleen declined with age, the PFC response in the mucosa-associated MLN did not decline with age but instead remained constant over the entire age span of 4 to 28 months studied. These studies showed that the spleen, peripheral lymph nodes, and MLN did not demonstrate parallel age-associated defects in antibody responses to pneumococcal polysaccharides when the antigen was administered systemically. Also, the deficient splenic antibody response to Pnu-Imune vaccine in aged mice could be enhanced by injecting a combination of Pnu-Imune vaccine and the nontoxic adjuvant monophosphoryl lipid A. Moreover, an immunoglobulin G response was induced when the immunogen was a mixture of vaccine and adjuvant.

The immune responses of mice to pneumococcal polysaccharide antigens are remarkably similar to those of humans in that both neonates and aged individuals are hyporesponsive to these antigens (9, 18, 22, 30, 31). Not surprisingly, there is an increased susceptibility of infants and aged humans to pneumococcal infections (18, 28, 36). A 23-valent pneumococcal polysaccharide vaccine was developed in 1983 to improve antibody responses in humans to encapsulated bacteria and was found to be protective in young adults but not in infants (8, 20). The efficacy of this vaccine in the elderly has been extremely controversial (29). Despite large immunization programs, bacterial pneumonia remains a serious cause of morbidity and mortality throughout the world and a significant cause of illness and death among the elderly. The mechanistic basis of this age-associated variation in immunity to polysaccharide antigens is not known. Further, the mucosal lymphoid apparatus, encompassing over a third of the body's lymphoid tissue and forming the first line of defense in infections, has recently been shown to retain immunocompetence in aged mice (33). This is interesting in the context of our recent findings which showed that a variety of type 2 thymus-independent antigens (TI-2) elicited excellent antibody responses from the spleen and mesenteric lymph nodes (MLN) but not from peripheral lymph nodes or lungs (15–17) and because pneumococcal polysaccharides have been considered TI-2 antigens (21).

To gain further insight into the effects of aging on the immune responses to pneumococcal polysaccharide antigens, we established a murine model system using the Pnu-Imune vaccine as the antigen. The polyvalent pneumococcal vaccine is a mixture of 23 capsular polysaccharides derived from the most commonly occurring serotypes of pneumococcal bacteria (12). In spite of extensive work with individual polysaccharides in the animal models, very little information is available about the characteristics of the immune responses of mice to this vaccine formulation. Such studies will be extremely important in improving the efficacy of this vaccine in children and the elderly. This work has focused on characterizing murine immune responses to the vaccine and analyzing the age-associated changes in such immune responses in systemic (spleen and peripheral lymph nodes) and mucosa-associated lymphoid organs (MLN). Procedures were developed to measure specific antibody response to the whole Pnu-Imune vaccine. The plaque-forming cell (PFC) response of spleen cells to pneumococal polysaccharides declined with age, whereas responses of the MLN increased or remained unchanged over a comparable age span. The data supports the hypothesis that B-cell responses of mucosal and systemic lymphoid organs diverge with age. Moreover, monophosphoryl lipid A [MPL(A)], a nontoxic adjuvant (26, 27), when given along with the Pnu-Imune vaccine, enhanced the immune responses to the vaccine in the spleens of aged mice and induced an immunoglobulin G (IgG) response to the vaccine.

MATERIALS AND METHODS

Mice. Female BALB/c-CRL mice of various ages, ranging from 4 to 28 months, were obtained from the National Institute of Aging (Bethesda, Md.) and the National Cancer Institute through Charles River Laboratories (Kingston, N.Y.). CB17 mice of age 5 to 16 months were bred in our animal colony.

Reagents. A 23-valent pneumococcal vaccine (i.e., Pnu-Imune 23) was obtained from Lederle Laboratories (Pearl

* Corresponding author.

River, N.Y.). MPL(A) (i.e., monophosphoryl lipid [MPL] plus trehalose dimycolate mixture emulsion) was purchased from RIBI Immunochemical Research Inc. (Hamilton, Mont.). Trinitrophenylated *Brucella abortus* (TNP-BA) was prepared as described before (17).

Immunization procedure. Mice were given a single intraperitoneal (i.p.) or subcutaneous injection of the optimal dose of Pnu-Imune vaccine (11.5 μg per mouse) or individual polysaccharide (0.5 μg per mouse) in saline alone or along with 25 μg of MPL(A) adjuvant. In these studies, the optimal dose of the vaccine was found to be the same for all the age groups of BALB/c mice examined. MPL(A) was reconstituted to 0.5 mg/ml in saline and mixed thoroughly to obtain an opalescent stock solution, which was stored at 4°C until use.

Cell preparation. Lymphoid cells to be assayed were obtained from the spleens, MLN, and peripheral lymph nodes, which included brachial, axillary, inguinal, cervical, popliteal, and periaortic lymph nodes. Adhering fat and connective tissues were removed from the lymphoid tissues. Lymphocytes were dispersed by pressing the spleens or lymph nodes against the bottom of a petri dish containing Hanks balanced salt solution using the flat surface of a sterile disposable syringe plunger. A mixture of serum-free Iscove's solution and Ham's F12 solution was supplemented with 10% fetal calf serum and was employed for all studies with lymph node cells (32). The spleens and lymph node cells were washed with Hanks balanced salt solution and were resuspended in the mixture of Iscove's and Ham's F12 solutions plus 10% fetal calf serum for assay.

Hemolytic PFC assay. The numbers of antibody-producing PFCs specific for pneumococcal polysaccharides were detected in individual mice. PFCs that made IgM antibodies were detected by a slide version of the technique of localized hemolysis in gel (19) by using indicator sheep erythrocytes (SRBC) coated with Pnu-Imune vaccine or individual polysaccharides or trinitrophenyl groups. The procedure described by Baker et al. (5) was employed to couple polysaccharides to SRBC. Briefly, this procedure consisted of washing the SRBC, coating with Pnu-Imune vaccine (1,000 μg/0.5 ml of packed SRBC) or individual polysaccharide (350 μg/0.5 ml of packed SRBC), and coupling with 1.0 ml of 0.1% freshly made chromium chloride solution. The suspension was rocked for 10 min at room temperature. The erythrocytes were then washed four times by centrifugation with 20 volumes of saline and adjusted to a final concentration of 10% (vol/vol) for use in the PFC assays. TNP-SRBC were prepared as described before (17).

Polyethylene glycol (average molecular weight, 7,500 to 8,000) (J. T. Baker Chemical Co., Phillipsburg, N.J.) was added to the reaction mixture containing 0.5% melted Sea-Plaque agarose (FMC Bioproducts, Rockland, Maine), lymphocytes, and antigen-coupled SRBC at a final concentration of 0.25% (wt/vol) to improve the clarity of the plaques. The number of PFCs on uncoupled SRBC was evaluated routinely and was found to be small (10 to 20 PFC/10^6 cells) and was subtracted from all experimental values of Pnu-Imune PFC reported here. PFC responses are expressed as the mean numbers (arithmetic means ± standard errors) of PFCs per lymphoid tissue for groups of three to six mice.

PFCs of the IgG isotype were detected by inhibiting the IgM plaques with a 1:100 dilution of goat anti-mouse IgM serum (Sigma Chemical Company, St. Louis, Mo.) which was added to the plaquing mixture and by facilitating the development of IgG1, IgG2, and IgG3 plaques, with the addition of predetermined optimal concentrations (1:80 dilu-

tion) of rabbit anti-mouse IgG1, IgG2, and IgG3 antisera, respectively (Nordic Immunological Laboratories, Tilburg, The Netherlands) to the plaquing mixture. This is known as indirect-plaque assay, a well-established procedure that detects all IgG isotypes irrespective of their ability to fix complement (16, 24).

Statistical analysis. The Student t test was used to assess the significance of the observed differences between groups. The differences were considered to be significant when the probability (P) value was <0.05.

RESULTS

Characterization of immune responses of mice to Pnu-Imune vaccine. A systematic analysis of doses, time periods, and various routes of immunization was performed to establish conditions for obtaining maximum response to the Pnu-Imune vaccine. Figure 1A summarizes the Pnu-Imune-specific response in the spleens and the MLN of BALB/c mice immunized i.p. with various doses of the vaccine. For both lymphoid organs, 11.5 μg of the vaccine was found to be the optimal dose that elicited the highest response. The PFC response peaked 5 days after i.p. immunization, and the peak responses in the spleen and MLN were obtained on the same day (Fig. 1B). The splenic response to i.p. challenge (636 ± 96 PFCs/10^6 cells) was always higher than that obtained upon subcutaneous immunization (341 ± 46 PFCs/10^6 cells) or intravenous immunization (140 ± 8 PFCs/10^6 cells). The MLN response was obtained only by i.p. immunization and was always less than the splenic response, possibly because i.p. immunization may not be the optimal route to elicit immune responses from this tissue.

The PFCs detected with the vaccine-coated SRBC were specific to Pnu-Imune vaccine, because the soluble vaccine inhibited the response completely, whereas only 10 to 20% of the response was inhibitable by cell wall polysaccharides or type 3 pneumococcal polysaccharide. The immunization procedure appeared to sensitize mice to almost all the polysaccharides, as determined by using SRBC coated with individual polysaccharides (21 of 23 were tested).

A sample of data (covering the whole spectrum of responses) with several polysaccharides is shown in Table 1. The response measured on vaccine-coated SRBC was 1,034 ± 141 PFCs/10^6 spleen cells, whereas the sum of responses measured on individual-polysaccharide-coated SRBC was 2,950 ± 130 PFCs/10^6 cells, suggesting that the former is on the average 33% as efficient in detecting the response to any individual component.

Differences in splenic and lymph node responses to Pnu-Imune. Previously we reported that TI-2 antigens like TNP-Ficoll elicited PFC responses from spleen and MLN B cells but not from draining lymph node B cells in the periphery (15, 17). To determine whether Pnu-Imune vaccine behaved like the other TI-2 antigens and whether MLN response can be obtained by another route of immunization, the vaccine was administered subcutaneously and the PFC response was measured in the peripheral lymph nodes (brachial, axillary, cervical, popliteal, inguinal, and periaortic) and MLN as well as in the spleen. Pnu-Imune vaccine did not elicit any PFC response in the peripheral lymph nodes and MLN but was effective in inducing a good splenic response (Fig. 2A). Although the experiment was performed with the optimal dose of the antigen (11.5 μg), other doses (higher and lower) of the vaccine were also ineffective in eliciting a PFC response from the peripheral lymph nodes. In the same experiment, TNP-BA, a TI-1 antigen, induced a good PFC
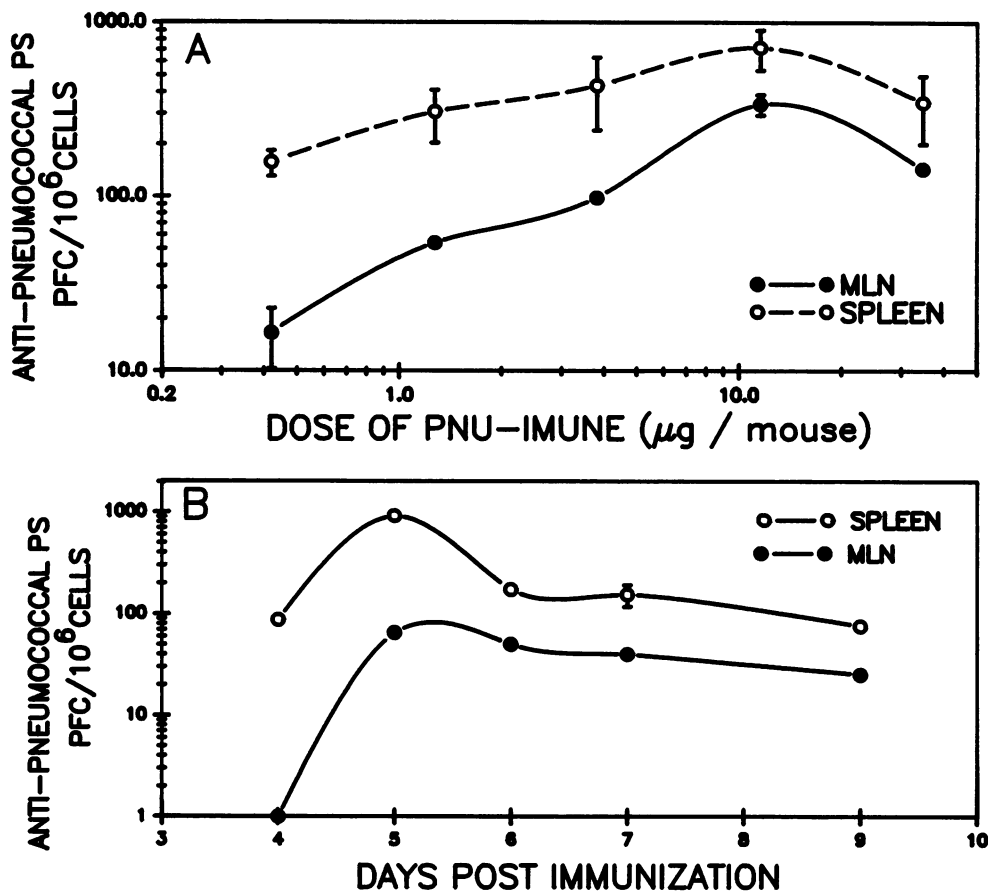
FIG. 1. (A) Immunogenicity of Pnu-Imune vaccine in mice. Young BALB/c mice (4 to 5 months old) were immunized i.p. with various doses of Pnu-Imune vaccine. Five days later, pneumococcal polysaccharide (PS)-specific PFCs were assayed from the spleens and MLN using SRBC coated with Pnu-Imune as indicator cells. (B) Kinetics of antibody response to Pnu-Imune vaccine in young BALB/c mice (4 to 5 months old) injected i.p. with the optimal dose (11.5 μg per mouse) of Pnu-Imune vaccine. The pneumococcal polysaccharide (PS)-specific PFC response from spleens and MLN was measured on days 4, 5, 6, 7, and 9 postimmunization. Each datum point represents the arithmetic mean ± standard error of the PFC response observed in three to five mice. In this and other figures, datum points without error bars represent data wherein the size of error bar was less than the size of the data point.

TABLE 1. Comparison of vaccine- and individual
polysaccharide-coated SRBC in detecting the
PFC response to Pnu-Imune

Coupling antigen	No. of PFCs/10^6 spleen cells (mean ± SE)
Pnu-Imune vaccine	1,034 ± 141
Pneumococcal type	
1	209 ± 64
5	113 ± 7
14	56 ± 2
22	45 ± 4
26	131 ± 4
57	94 ± 16
70	35 ± 8

[a] Young BALB/c mice (4 to 5 months of age) were immunized with 11.5 μg of Pnu-Imune vaccine. The PFC response was measured on day 5, using SRBC coupled with vaccine or indicated polysaccharides (U.S. nomenclature used for pneumococcal types). Sum of responses measured using SRBC coated individually with 21 of the polysaccharides was 2,950 ± 130 PFCs/10^6 spleen cells.

response from these draining lymph nodes (Fig. 2B), in agreement with our previous results (17). Four polysaccharides (types 1, 3, 5, and 19) were tested individually for the dichotomy of response from the spleens and lymph nodes. Consistent with the data from the whole vaccine, none of the polysaccharides elicited a measurable response from the peripheral lymph nodes. In the same experiment, the splenic responses were 1,125 ± 124, 923 ± 143, 1,208 ± 121, and 821 ± 220 PFCs/10^6 cells for type 1, 3, 5, and 19 polysaccharides, respectively.

Effect of age on murine immune response to Pnu-Imune vaccine. BALB/c mice (22 to 24 month old) were immunized i.p. with various doses of Pnu-Imune vaccine, and the antibody responses were measured 5 days later (Fig. 3A). Just as in young mice, 11.5 μg continued to be the optimal dose for aged mice, and the peak response was obtained on day 5 (Fig. 3B). Similarly, the PFC response to an i.p. challenge (181 ± 7 PFCs/10^6 cells) was higher than to subcutaneous immunization (120 ± 12 PFCs/10^6 cells) with the vaccine. To determine the effect of age on the immune response to the vaccine, various age groups of BALB/c mice were immunized i.p. with the optimal dose (11.5 μg/mouse) of the Pnu-Imune vaccine. Results shown in Fig. 3C demonstrated clearly that splenic immune responses to Pnu-
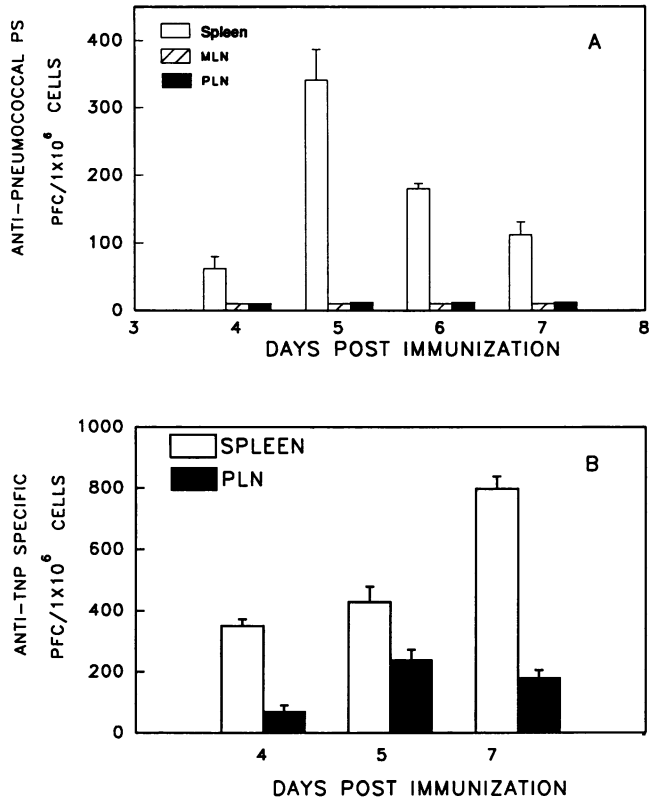
FIG. 2. (A) Unresponsiveness of peripheral lymph nodes to the Pnu-Imune vaccine. The optimal dose (11.5 µg per mouse) of Pnu-Imune vaccine was administered subcutaneously to young BALB/c mice (4 to 5 months old) in the left armpit near the shoulder area and into footpads. The pneumococcal polysaccharide (PS)-specific PFC response in the spleens, MLN, and peripheral lymph nodes (PLN) (brachial, axillary, cervical, popliteal, inguinal, and periaortic) was assayed at different times after immunization. Each datum point represents the arithmetic mean ± standard error of PFC response observed in four to five mice. (B) The optimal dose of 50 µl of 1:10 dilution of stock TNP-BA per mouse was administered subcutaneously to young BALB/c mice. PFC response was assayed for the spleens and peripheral lymph nodes at days 4, 5, and 7.

Imune vaccine declined steadily with increasing age of the mice. The maximum splenic response occurred in the 4- to 5-month-old mice, and 22-month-old mice exhibited responses which were significantly less ($P < 0.001$) than the maximum value. The vaccine failed to elicit antibody response from peripheral lymph nodes from mice of all age groups. Interestingly, PFC responses in the mucosa-associated lymph nodes did not decline but instead remained relatively constant over the entire age span studied. In this study, mice younger than 4 months were not tested, but other studies with individual polysaccharides demonstrated that younger mice had reduced responses (21, 22).

To determine whether senescence had any positive effects on the immune responses of peripheral lymph nodes to the Pnu-Imune vaccine, we immunized 22- to 24-month-old BALB/c mice subcutaneously with this vaccine in the left armpit near the shoulder and into the foot pads. As shown in Table 2, 22- to 24-month-old mice behaved like young mice and did not show any response in the peripheral or MLN. These lymph node cells responded well to challenge with TNP-BA, consistent with our previous studies on lymph
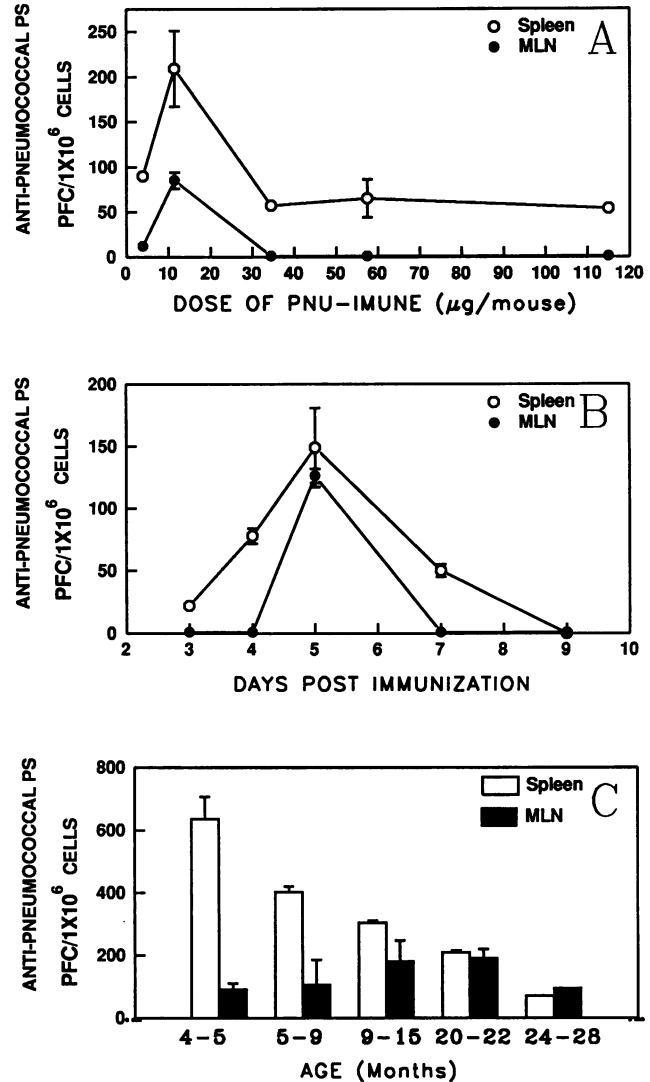
FIG. 3. (A) Dose dependency of Pnu-Imune response in aged mice. Various doses (3.9, 11.5, 34.5, 57.6, and 115 µg per mouse) of vaccine were injected i.p. into 22- to 24-month-old BALB/c mice. PFC response was measured at day 5 for the spleens and MLN. (B) Kinetics of Pnu-Imune response in aged mice. The optimal dose of 11.5 µg of vaccine was injected i.p. into 22- to 24-month-old BALB/c mice, and the PFC response was measured on days 3, 4, 5, 7, and 9. (C) Effect of age on the immune response to the Pnu-Imune vaccine. Indicated age groups of BALB/c mice were immunized i.p. with the optimal dose (11.5 µg per mouse) of the Pnu-Imune vaccine. After 5 days, the PFC response was assayed for the spleens, MLN, and peripheral lymph nodes. The vaccine failed to elicit any detectable response from peripheral lymph nodes. Each datum point represents the arithmetic mean of PFC response observed in four to five mice. The error bars represent standard errors of the mean. PS, polysaccharide.

node responses to such antigens (15, 17). Although there was a detectable splenic response to Pnu-Imune in these aged mice, it was small compared with that of young mice.

The nontoxic adjuvant, MPL(A), enhances the immune response to the vaccine. Young mice (4 to 5 months old) and old mice (22 to 24 months old) were immunized i.p. with various doses of MPL(A) along with an optimal dose of the Pnu-Imune vaccine. Twenty-five micrograms of MPL(A)

TABLE 2. PFC responses to Pnu-Imune vaccine in the spleens, MLN, and peripheral lymph nodes of 22- to 24-month-old BALB/c mice[a]

Antigen and site of injection	No. of PFCs/10^6 cells[b]		
	Spleen	Peripheral lymph nodes[c]	MLN
Pnu-Imune			
(Near shoulder)	91 ± 32	NR	NR
(Footpads)	120 ± 12.4	NR	27 ± 3
TNP-BA	167 ± 64	94 ± 2.8	ND

[a] The optimal dose (11.5 μg per mouse) of Pnu-Imune vaccine or TNP-BA (50 μl of 1:10 dilution of the stock) was administered subcutaneously in the left armpit near the shoulder area as well as into footpads. Antibody response was measured 5 days later, using vaccine- or TNP-coated SRBC.

[b] The arithmetic mean ± standard error of the PFC responses from four mice is shown. NR, no response; ND, not done.

[c] These include brachial, axillary, cervical, popliteal, inguinal, and periaortic lymph nodes.

FIG. 4. (A) Effect of the adjuvant MPL(A) on the Pnu-Imune responses of young and old mice. The optimal dose (11.5 μg per mouse) of Pnu-Imune vaccine along with 25 μg of MPL(A) per mouse was administered i.p. to young (4- to 5-month-old) and old (22- to 24-month-old) BALB/c mice, and 5 days later the pneumococcal polysaccharide (PS)-specific PFC response was evaluated for spleens and MLN. Each datum point represents the arithmetic mean ± standard error of the PFC responses of three to five mice. (B) Lack of adjuvant effect of MPL(A) on the peripheral lymph node response to the Pnu-Imune vaccine. The optimal dose (11.5 μg per mouse) of Pnu-Imune vaccine along with 25 μg of MPL(A) per mouse was administered to young (4- to 5-month-old) and old (22- to 24-month-old) BALB/c mice subcutaneously. Five days later, the pneumococcal polysaccharide (PS)-specific PFC response was assayed for the spleens, MLN, and draining lymph nodes (brachial, axillary, cervical, inguinal, popliteal, and periaortic). The arithmetic means ± standard errors of PFC responses of four to five mice are plotted. For peripheral lymph nodes (PLN), only the response in the presence of MPL(A) plus vaccine, which is very small, is shown. MPL(A) alone or the vaccine alone failed to induce any response in the lymph nodes.

was found to be optimal in enhancing the Pnu-Imune response in young and old mice, while smaller doses were ineffective as an adjuvant and higher doses of MPL(A) (100 μg) were inhibitory in both young and old mice. At the optimal dose, the MPL(A) adjuvant enhanced the antibody response from the spleens of old mice (22 to 24 months) by a factor of 10 (Fig. 4A), but there was little or no response in mice immunized only with the adjuvant. The splenic response of young mice was also enhanced by MPL(A) but only by a factor of about 3. The response in MLN was enhanced by MPL(A) only in young mice. Since, in humans, the i.p. route is not practical, we tested the ability of MPL(A) to be used as an adjuvant if administered via the subcutaneous route. The splenic response increased when Pnu-Imune vaccine was administered subcutaneously along with the MPL(A) adjuvant (Fig. 4B). The MPL(A)-induced enhancement was greater in old mice than in young mice. However, MPL(A) was unable to induce any Pnu-Imune response in the draining lymph nodes of young or old mice. Injection of MPL(A) 2 days after subcutaneous immunization with the optimal dose (11.5 μg) of Pnu-Imune vaccine caused a slight increase in the numbers of IgM-secreting pneumococcal polysaccharide-specific PFC from peripheral draining lymph nodes (0 [without MPL] versus 32 ± 5 PFCs/10^6 cells [with MPL]). In contrast, subcutaneous administration of MPL(A) induced a substantial enhancement of the IgM antibody response from the spleen. This increase was similar in magnitude to those noted in other experiments such as those shown in Fig. 4A in which MPL(A) was injected along with the Pnu-Imune vaccine.

The Pnu-Imune vaccine alone induced an IgM PFC response, but no IgG response, in young and old mice. Administration of MPL(A) along with the vaccine induced IgG3 responses in both age groups (Table 3). Yet there was no IgG1 or IgG2 response, suggesting that the response observed with anti-IgG antibodies is mainly due to the IgG3 component. The isotype distribution of the response remained the same at later times after immunization. Notably, the enhancing effect of MPL(A) on the Pnu-Imune response in the spleen could be observed even at day 11 in 22-month-old mice (Fig. 5) as well as in 6- to 9-month-old mice (data not shown). The response became undetectable by day 15.
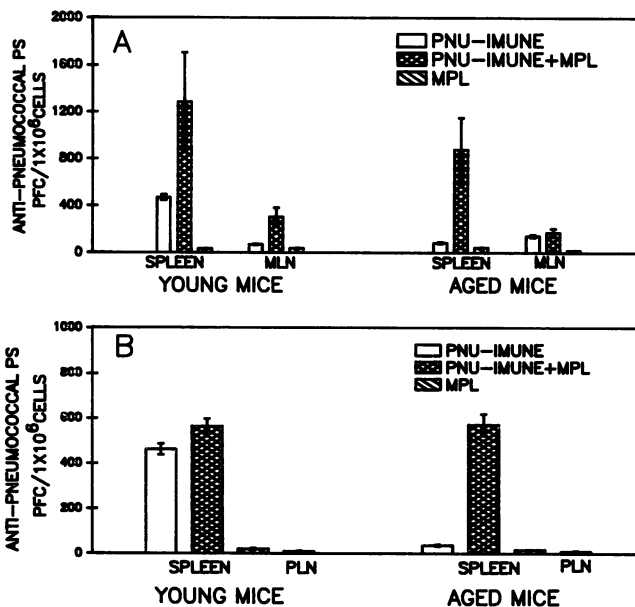
DISCUSSION

An animal model system was developed to study the effect of age on immune responses to the vaccine containing pneumococcal polysaccharides. The commercially available Pnu-Imune vaccine was found to be effective in inducing a good antibody-forming cell response in the murine system. The optimal dose requirements and the peak of the response for the vaccine were similar to those observed with the individual polysaccharides in mice (4). In experiments not shown here, it was demonstrated that immunization with the vaccine elicited measurable antibody responses against 21 polysaccharides present in the vaccine (14a).

Study of the immune responses in different lymphoid organs demonstrated that the splenic and MLN B cells, but not B cells from peripheral lymph nodes, responded well to the vaccine. In this, the vaccine behaved like TNP-Ficoll and other TI-2 antigens, as reported by us earlier (15–17). Further, MLN responses were obtained only by i.p. challenge but not subcutaneous challenge with the vaccine (data not shown), whereas the splenic response was induced by either mode of immunization. These results provide an

TABLE 3. Effect of adjuvant MPL(A) on the isotype distribution of the PFC response to Pnu-Imune vaccine[a]

Antigen	Age (mo) of mice	No. of PFCs/10⁶ spleen cells[b]				
		IgM	IgG	IgG1	IgG2	IgG3
Pnu-Imune	5 to 6	551 ± 40	0	0	0	0
Pnu-Imune + MPL(A)	5 to 6	838 ± 2	140 ± 11	0	0	201 ± 13
Pnu-Imune	16	455 ± 8	0	0	0	0
Pnu-Imune + MPL(A)	16	867 ± 20	124 ± 21	0	0	186 ± 14
Pnu-Imune	22	149 ± 32	0	0	0	0
Pnu-Imune + MPL(A)	22	638 ± 14	250 ± 32	0	0	180 ± 6

[a] The optimal dose (11.5 μg per mouse) of Pnu-Imune vaccine along with 25 μg mouse of MPL(A) was administered i.p. to 5- to 6-, 16-, and 22-month-old BALB/c mice. The PFC response was measured 5 days after immunization. Isotypes of the PFCs were determined as described in Materials and Methods. Administration of MPL(A) alone failed to elicit any IgM or IgG PFC response.
[b] The values represent the arithmetic means ± standard errors of responses of two mice.

explanation for the well-known splenic dependency of murine and human immune responses to pneumococcal polysaccharides (2, 7). Our previous studies with other TI-2 antigens indicated that a splenic accessory cell was required to induce antibody response to TNP-Ficoll in the peripheral lymph nodes (17). Currently, we are investigating the role of such accessory cells in the immune responses of peripheral lymph nodes to the Pnu-Imune vaccine.

As seen in aged humans, the immune response to the Pnu-Imune vaccine also decreased steadily with increasing age of the mouse. A similar age-associated decline in responsiveness of the spleen to type III polysaccharide was reported previously by Smith (31). However, the age-associated decline was limited to the splenic response. The MLN B cells from aged mice were as effective as those from young mice in eliciting good immune responses to the Pnu-Imune vaccine. These findings are consistent with the hypothesis that the mucosa-associated lymphoid system differs from the systemic immune system with regard to its competence with age (33). Thus, Wade and Szewczuk (40) found that antibody responses of MLN B cells to T-dependent antigens did not decrease with age. In addition, Thoman and Weigle (34) reported that T-cell proliferation and interleukin 2 (IL-2) secretion were well preserved in MLN T cells, while their splenic counterparts exhibited age-associated defects. From studies of lymphocyte trafficking and homing, it has been

proposed that the mucosa-associated lymphoid system contains a distinct lymphoid population (1, 6, 11, 23). Indeed, the homing receptors for Peyer's patches and peripheral lymph nodes have been found to be distinct (14). The reason why the mucosa-associated lymphoid system remains immunologically vigorous at a time when systemic immunity declines is not known but may be related to the constant environmental stimulation present in the mucosal tissues. For practical purposes, it would be valuable if an immunization protocol could recruit this system to obtain a good antibody response to the Pnu-Imune vaccine in aged humans. Currently, we are attempting oral immunization with the vaccine and supplementation with adjuvants to stimulate the mucosal immune system of mice.

Studies by Tomai et al. (35) showed that MPL(A) was a good adjuvant in restoring age-dependent losses in immune responses to SRBC. Later Baker et al. (3) reported that MPL(A) also enhanced the responses of young mice to type III pneumococcal polysaccharides and suggested that MPL(A) may be effective in overcoming the effect of suppressor cells. Even though we do not know whether the age-associated decline in responsiveness to Pnu-Imune vaccine is a result of suppressor function, we found that MPL(A) substantially enhanced the antibody response of aged mice to the Pnu-Imune vaccine. Administration of Pnu-Imune vaccine along with MPL(A) induced a response which remained elevated up to day 11. Also, the adjuvant induced antibodies of IgG and IgG3 subtypes, which usually have longer half-lives than IgM (37), and thus may prolong protective immunity. Since IgG responses are usually associated with memory, supplemental immunization with the adjuvant might provide an opportunity to obtain secondary responses to the Pnu-Imune vaccine. We are currently investigating this possibility. These results are extremely encouraging, since large doses of MPL(A) have been found to be relatively nontoxic to humans in phase I clinical trials (39).

Several factors may contribute to the ability of MPL(A) to act as an adjuvant in enhancing the immune response and inducing an isotype switch. First, MPL(A) is mitogenic to B cells and thus may act synergistically with antigen in stimulating B cells (25). Second, MPL(A) plus trehalose dimycolate mixture activates macrophages and stimulates the production of IL-1 (10, 13, 26, 27, 41). Our studies with TNP-Ficoll, another TI-2 antigen showed that splenic accessory cells or IL-1 could restore lymph node B-cell responses to TI-2 antigens (17). The mechanisms by which MPL(A) induces an IgG response to the vaccine is unknown. The IgG responses are very much dependent on the type of T helper cells (TH1 versus TH2) induced and the production of
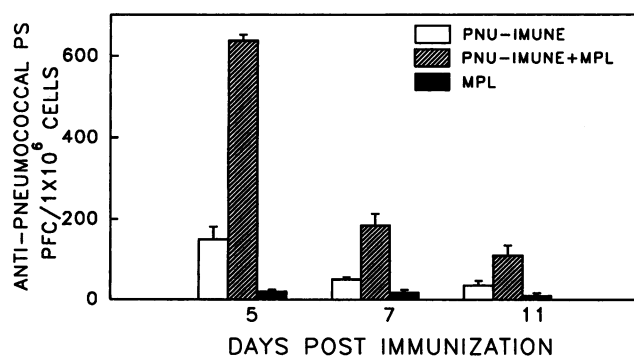

FIG. 5. The adjuvant MPL(A) prolongs the immune response to the Pnu-Imune vaccine. Old BALB/c mice (22 to 24 months old) were immunized i.p. with 11.5 μg of the Pnu-Imune vaccine per mouse along with 25 μg of MPL(A). The antibody responses were analyzed on days 5, 7, 11, and 15 postimmunization. The response on day 15 was too little to measure and therefore was not shown. Each datum point represents the arithmetic mean of the PFC response observed in four to five mice. The error bars represent the standard errors of the mean.

specific cytokines such as IL-4, gamma interferon, transforming growth factor β, etc. (38). Conceivably, MPL(A) is either inducing such a T-cell helper or stimulating the production of relevant cytokines from other accessory cells. Currently, we are evaluating the specific subtypes of IgG and IgA that may be induced in this response.

In summary, we have shown that murine immune responses to the Pnu-Imune vaccine declined with age as in humans and could be enhanced with the adjuvant MPL(A). The adjuvant was also effective in inducing an IgG response to the vaccine. The preservation of Pnu-Imune responses in the MLN cells of the elderly suggests that alternative strategies of immunization with the vaccine should be explored.

ACKNOWLEDGMENTS

REFERENCES

1. Allardyce, R. A., and J. Bienenstock. 1984. The mucosal immune system in health and disease with an emphasis on parasitic infection. Bull. W.H.O. 62:7–25.
2. Amlot, P. L., D. Grennan, and J. H. Humphrey. 1985. Splenic dependence of the antibody response to thymus independent (TI-2) antigens. Eur. J. Immunol. 15:508–512.
3. Baker, P. J., J. R. Hiernaux, M. B. Fauntleroy, P. W. Stashak, B. Prescott, J. L. Cantrell, and J. A. Rudbach. 1988. Ability of monophosphoryl lipid A to augment the antibody response of young mice. Infect. Immun. 56:3064–3066.
4. Baker, P. J., P. W. Stashak, D. F. Amsbaugh, and B. Prescott. 1971. Characterization of the antibody response to type III pneumococcal-polysaccharide at the cellular level. I. Dose response studies and the effect of prior immunization on the magnitude of the antibody response. Immunology 20:469–480.
5. Baker, P. J., P. W. Stashak, and B. Prescott. 1969. The use of erythrocytes sensitized with purified pneumococcal polysaccharides for the assay of antibody and antibody producing cells. Appl. Microbiol. 17:422–426.
6. Bienenstock, J. 1984. The mucosal immunological network. Ann. Allergy 53:535–540.
7. Bohnsack, J. F., and E. J. Brown. 1986. The role of the spleen in resistance to infection. Annu. Rev. Med. 37:49–59.
8. Bolan, G., C. V. Broome, R. R. Facklam, B. D. Plikaytis, D. W. Fraser, and W. F. Schlech III. 1986. Pneumococcal vaccine efficacy in selected populations in the United States. Ann. Intern. Med. 104:1–6.
9. Callard, R. E., A. Basten, and L. K. Waters. 1977. Immune function in aged mice. II. B-cell function. Cell. Immunol. 31:26–36.
10. Carozzi, S., M. Salit, A. Cantaluppi, M. G. Nasini, S. Barocci, S. Cantarella, and S. Lamperi. 1989. Effect of monophosphoryl lipid A on the in vitro function of peritoneal leukocytes from uremic patients on continuous ambulatory peritoneal dialysis. J. Clin. Microbiol. 27:1748–1753.
11. Cebra, J. J., P. J. Gearhart, and R. Kamat. 1977. Origin and differentiation of lymphocytes involved in the secretory IgA responses. Cold Spring Harbor Symp. Quant. Biol. 41:201–215.
12. Centers for Disease Control. 1984. Update: pneumococcal polysaccharide vaccine usage. United States. Morbid. Mortal. Weekly Rep. 33:273–281.
13. Dijkstra, J., J. W. Mellors, J. L. Ryan, and F. C. Szoka. 1987. Modulation of the biological activity of bacterial endotoxin by incorporation into liposomes. J. Immunol. 138:2663–2670.
14. Gallatin, W. M., I. L. Weissman, and E. C. Butcher. 1983. A cell surface molecule involved in organ specific homing of lymphocyte. Nature (London) 304:30–34.
14a. Garg, M., and B. Subbarao. Unpublished data.
15. Goud, S. N., A. M. Kaplan, and B. Subbarao. 1990. Antibody response to thymic independent-antigens in the peripheral and mesentric lymph nodes of mice. Reg. Immunol. 3:1–7.
16. Goud, S. N., A. M. Kaplan, and B. Subbarao. 1990. Primary antibody responses to thymus-independent antigens in the lung and hilar lymph nodes of mice. Infect. Immun. 58:2035–2041.
17. Goud, S. N., N. Muthusamy, and B. Subbarao. 1988. Differential responses of B cells from the spleen and lymph node to TNP-Ficoll. J. Immunol. 140:2925–2930.
18. Gray, B. M., and H. C. Dillon. 1986. Clinical and epidemiologic studies of pneumococcal infection in children. Pediatr. Infect. Dis. J. 5:201–207.
19. Jerne, N. K., and A. A. Nordin. 1963. Plaque formation in agar by single antibody producing cells. Science 140:405.
20. Laforce, F. M., and T. C. Eicknoff. 1988. Pneumococcal vaccine: an emerging consensus. Ann. Int. Med. 108:757–759.
21. Mosier, D. E., and B. Subbarao. 1982. Thymus-independent antigens: complexity of B lymphocyte activation revealed. Immunol. Today 3:217–222.
22. Mosier, D. E., N. M. Zaldivar, and E. Goldings. 1977. Formation of antibody in the newborn mouse: study of T-cell independent antibody. J. Infect. Dis. 136:S14–S19.
23. Orga, P. L., and R. Dhar. 1985. Local immune responses. Br. Med. Bull. 41:28–32.
24. Pasanen, V. J., R. Asofsky, and P. J. Baker. 1979. Synthesis of two classes of antibody, IgM and IgG, or IgM and IgA by identical cells. Amplification of the antibody response to pneumococcal polysaccharide type III. J. Exp. Med. 149:1227–1237.
25. Ribi, E., J. L. Cantrell, K. Takayama, N. Qureshi, J. Peterson, and H. O. Ribi. 1984. Lipid A and immunotherapy. Rev. Infect. Dis. 6:567–572.
26. Ribi, E., J. L. Cantrell, K. Takayama, H. O. Ribi, K. R. Myers, and N. Qureshi. 1986. Modulation of humoral and cell mediated immune responses by a structurally established nontoxic lipid A, p. 407–420. In A. Szentivanji and H. Friedman (ed.), Immunobiology and immunopharmacology of bacterial endotoxins. Plenum Press, New York.
27. Ribi, E., J. T. Ulrich, and K. N. Masihi. 1987. Immunopotentiating activities of monophosphoryl lipid A, p. 101–112. In J. Majde (ed.), Immunopharmacology of infectious diseases: vaccine adjuvants and modulators of non-specific resistance. Alan R. Liss, Inc., New York.
28. Roghmann, K. J., P. A. Tablaski, D. W. Bentley, and G. Schiffman. 1987. Immune response of elderly adults to pneumococcus: variation by age, sex, and functional impairment. J. Gerontol. 42:265–270.
29. Sims, R. V., W. C. Steinmann, J. H. McConville, L. R. King, W. C. Zwick, and J. S. Schwartz. 1988. The clinical effectiveness of pneumococcal vaccine in the elderly. Ann. Intern. Med. 108:653–657.
30. Siskind, G. W., P. Y. Peterson, and L. Thomas. 1963. Induction of unresponsiveness and immunity in newborn and adult mice with pneumococcal polysaccharide. J. Immunol. 90:929–934.
31. Smith, A. M. 1976. The effects of age on the immune response to type III pneumococcal polysaccharide and bacterial lipopolysaccharide in BALB/c, SJL/J and C3H mice. J. Immunol. 116:469–474.
32. Subbarao, B., and D. E. Mosier. 1983. Induction of B lymphocyte proliferation by monoclonal anti-Lyb2 antibody. J. Immunol. 130:2033–2037.
33. Szewczuk, M. R., R. J. Campbell, and L. K. Jung. 1981. Lack of age associated immune dysfunction in mucosal associated lymph nodes. J. Immunol. 126:2200–2204.
34. Thoman, M. L., and W. O. Weigle. 1989. The cellular and subcellular bases of immunosenescence. Adv. Immunol. 46:221–261.
35. Tomai, M. A., L. E. Solem, A. G. Johnson, and E. Ribi. 1987. The adjuvant properties of a nontoxic monophosphoryl lipid A in hyporesponsive and aging mice. J. Biol. Response Modif. 6:99–107.
36. Verghese, A., and S. L. Berk. 1983. Bacterial pneumonia in the

elderly. Medicine (Baltimore) **62**:271–285.

37. **Vieiza, P., and K. Rajewsky.** 1988. The half lives of serum immunoglobulins in adult mice. Eur. J. Immunol. **18**:313–316.

38. **Vitetta, E. S., M. T. Berton, C. Burger, M. Kepron, W. T. Lee, and X.-M. Yin.** 1991. Memory B and T cells. Annu. Rev. Immunol. **9**:143–218.

39. **Vosika, G. J., C. Barr, and D. Gilbertson.** 1984. Phase-1 study of intravenous modified lipid A. Cancer Immunol. Immunother. **18**:107–112.

40. **Wade, A. W., and M. R. Szewczuk.** 1988. Evidence for the divergence of systemic and mucosal B-cells with age in the primary and secondary immune responses to T-dependent antigens: route of priming influences isotype profile of antigen specific memory and antibody secreting B-cells. Aging Immunol. Infect. Dis. **1**:99–119.

41. **Yarkoni, E., L. Wang, and A. Bekierkunst.** 1977. Stimulation of macrophages by cord factor and by heat-killed and living BCG. Infect. Immun. **16**:1–8.