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Reversal of Age-Associated Decline in Immune Response to Pnu-Imune Vaccine by Supplementation with the Steroid Hormone Dehydroepiandrosterone

MANJU GARG AND SUBBARAO BONDADA*

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

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Recently, we reported that murine antibody responses to the 23-valent pneumococcal polysaccharide (Pnu-Imune) vaccine declined with age. Here we present data to support the concept that age-associated immune defects are not only due to intrinsic defects in immune cells but are also due to extrinsic factors emanating from the neuroendocrine system. We found that supplementation with dehydroepiandrosterone, a steroid hormone known to be reduced in the aged, corrects the immune deficiency of aged mice and significantly enhanced their splenic immune responses to the Pnu-Imune vaccine.

Pneumococcal infections remain an important cause of morbidity and mortality in the United States, particularly in children less than 2 years of age, in elderly persons, and in patients with AIDS (2, 10, 11). To prevent these infections, a vaccine made up of capsular polysaccharides from 23 commonly occurring pneumococcal bacteria was developed and was shown to reduce significantly the occurrence of pneumonia in a young healthy population (12). However, the vaccine was not protective in infants and was only moderately effective in the aged population (12, 19) and therefore needs to be improved. The recently described increase in the incidence of antibiotic-resistant strains of pneumococcal bacteria further emphasizes the need to make the current vaccine more efficacious (16). We developed a model system to study the immune responses of mice to pneumococcal polysaccharide (PS) antigens present in the commercial vaccines and showed that the splenic antibody responses to the 23-valent Pnu-Imune vaccine declined with increasing age (6).

In vivo, the immune microenvironment is a system composed of many components derived from the immune, nervous, and endocrine systems. Although immune responses can be elicited from isolated lymphocyte populations, there is growing evidence to suggest that factors of neural and endocrine origins can influence immune cell function (18). Recently, a newer concept was proposed to explain immunosenescence; i.e., the age-associated decline in immune function is due not only to loss of immune cell function (7, 13, 17, 20, 22) but also to altered endocrine status during aging (3). Thus, changing the milieu of steroid hormones such as glucocorticoids, dehydroepiandrosterone (DHEA), testosterone, and dihydroxytestosterone was shown to change the pattern and quantity of lymphokine production by T cells and thus affect immune responses in vivo (1, 4, 5).

DHEA and its precursor, DHEA-sulfate (DHEA-S), are made by adrenal glands, and their levels decrease with increasing age in both males and females (15). Daynes and Araneo showed that an exogenous supply of DHEA-S to aged animals reversed the age-associated abnormal secretion of lymphokines from T cells (decrease in interleukin-2 [IL-2] and increase in IL-4, IL-5, and gamma interferon) and especially enhanced the IL-2 production and the antibody responses to protein antigens (3).

Since the in vitro immune response to several type 2 thymus-independent (TI) antigens and to the vaccine is enhanced by T-cell-derived lymphokines (5a, 8, 9) and since DHEA was shown to restore T-cell function in the aged mice (1, 3), we studied the effect of DHEA-S therapy on the immune responses of aged mice to the pneumococcal vaccine. Short-term treatment with DHEA or DHEA-S significantly enhanced the antibody-forming cell responses of old but not young mice to the Pnu-Imune vaccine.

Female CB-17 mice were bred and housed in our animal colony. Female BALB/c-CRL mice were obtained from the National Institute of Aging (Bethesda, Md.). Young mice were 4 to 5 months old. Aged mice were 22 to 24 months old in the case of BALB/c mice, while the CB-17 mice were 15 to 17 months old. The 23-valent Pnu-Imune 23 vaccine was obtained from Lederle Laboratories (Pearl River, N.Y.). DHEA and DHEA-S (Sigma Chemical Co., St. Louis, Mo.) were dissolved in 95% ethanol and in propylene glycol, respectively. Initially, DHEA-S was used since it is not as rapidly degraded as DHEA (3, 15). The sulfate form of the hormone is taken up by the macrophages and converted into hormone and is released into the local environment and circulation, because of which higher local concentrations of hormone are obtained with DHEA-S than with DHEA in lymphoid tissues (4, 5).

Lymphoid cells were obtained from spleen, mesenteric lymph nodes (MLN), and draining peripheral brachial and axillary lymph nodes (PLN) and were processed as described previously (6, 9). The numbers of immunoglobulin M-producing plaque-forming cells (PFC) specific for PS were detected in individual mice with sheep erythrocytes (SRBC) coated with Pnu-Imune vaccine as described earlier (6). Previously, we showed that 11.5 μg is the optimal vaccine dose for both young and old mice, that the responses peaked on day 5, and that this method detected antibody responses to 21 of 23 component polysaccharides in the vaccine (6). Our previous studies found that 80 to 90% of the PFC response was specific to the capsular polysaccharides whereas 10 to 20% of the response was directed against the cell wall polysaccharide, which is a common contaminant in

* Corresponding author.

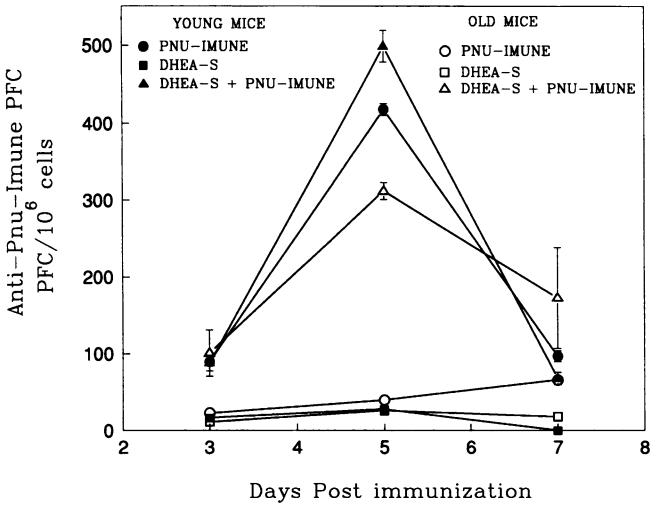

FIG. 1. Young (4 to 5 months) and old (15 to 17 months) CB-17 mice were injected s.c. with 100 μg of DHEA-S or vehicle alone, and 3 h later they were immunized at the same site with 11.5 μg of Pnu-Imune vaccine, a dose shown to be optimal for both young and aged mice in our previous studies (6). On days 3, 5, and 7, the Pnu-Imune-specific PFC were assayed by using vaccine-coupled SRBC and uncoupled SRBC as described in the text. The data represent mean responses of three to five mice, and the bars represent SE values. Representative results from one of three experiments are presented.

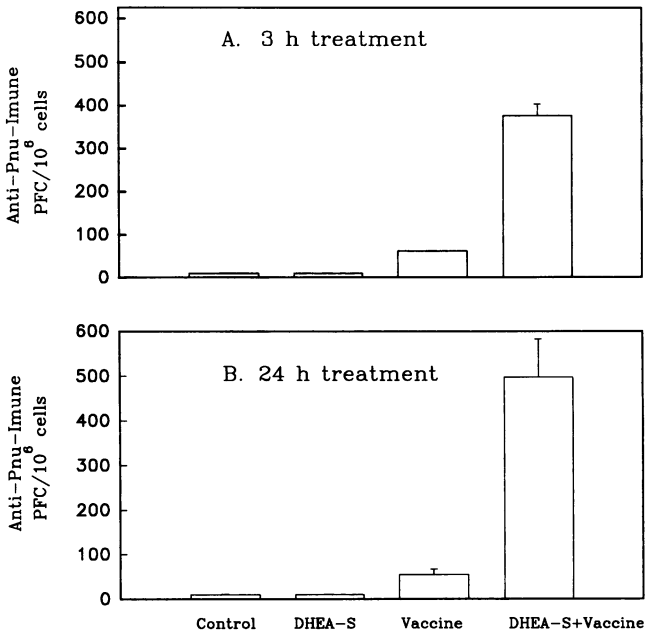

FIG. 2. DHEA-S was administered to old (22 to 24 months) BALB/c mice as described in the legend to Fig. 1, but the vaccine was injected 3.0 (A) or 24 (B) h later at the same site. Five days later, levels of Pnu-Imune-specific PFC were determined. Results represent mean responses of three to five mice per group, and the bars represent SE values. Similar results were obtained in another experiment.

capsular polysaccharides (6). The number of PFC on uncoupled SRBC was evaluated routinely (10 to 20 PFC per 10^6 cells) and was subtracted from all experimental values. Each control and experimental group consisted of three to five mice, and the values reported represent the arithmetic mean ± standard error (SE). The differences between control and DHEA-treated groups were evaluated by Student's *t* test. *P* values of less than 0.05 were considered significant.

To enhance the antibody responses of aged mice to the Pnu-Imune vaccine, 100 μg of DHEA-S per mouse or the vehicle was injected subcutaneously (s.c.) near the shoulder area of young as well as aged CB-17 mice. Three hours later, an optimal dose of Pnu-Imune vaccine was injected s.c. at the same skin site. As shown clearly in Fig. 1, a local injection of DHEA-S prior to immunization with the vaccine enhanced the splenic responses of aged mice to the Pnu-Imune vaccine on all days assayed but the peak response was on day 5. In young mice, DHEA-S therapy has only a moderate influence on the antibody responses to the vaccine. The hormone treatment did not increase the size of the spleen, and the results were similar if expressed as PFC per spleen. As reported previously, the draining PLN did not respond to the vaccine (6), and the hormone treatment had modestly increased their response (0 PFC in control versus 25 PFC per 10^6 cells in DHEA-injected mice). To determine whether DHEA-S will be effective at later time points, aged mice were given DHEA-S as described above and then were challenged s.c. with the Pnu-Imune vaccine 3 or 24 h later. Both 3 (Fig. 2A) and 24 (Fig. 2B) h of DHEA-S therapy were equally effective in significantly enhancing the Pnu-Imune-specific responses from aged mice compared with those from control mice that were not treated with the hormone.

Since DHEA-S requires the action of a sulfatase to generate physiologically active DHEA, a step that could differ in young and old mice, we tested the effect of DHEA itself on the vaccine response. Young and old BALB/c mice were given DHEA hormone near the shoulder region, and 3 h later an optimal dose of the vaccine was given s.c. Antibody responses from the spleen and draining PLN were assayed on day 5, and the results shown in Fig. 3 demonstrated that direct administration of DHEA also augmented the splenic PFC responses from old mice to Pnu-Imune vaccine. Once again, the hormone had only a very modest effect on the vaccine responses of young mice, suggesting that the lack of effect with DHEA-S in the young was not due to a difference

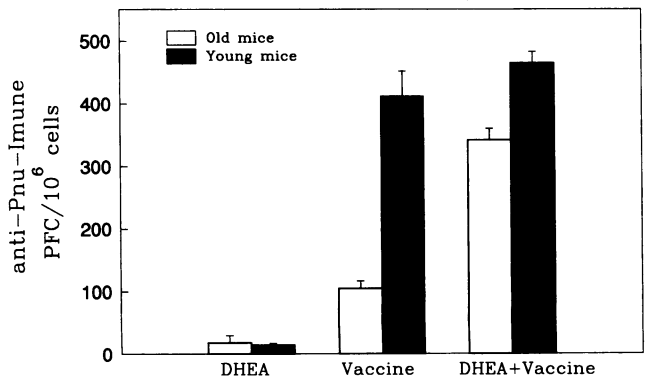

FIG. 3. The hormone DHEA (10 μg) in 95% ethanol or the vehicle alone was injected s.c. into young (4 to 5 months) or old (22 to 24 months) BALB/c mice, and 3 h later mice were immunized with 11.5 μg of Pnu-Imune vaccine. The vaccine-specific PFC were measured on day 5, when the Pnu-Imune response was previously found to be maximum (Fig. 1) (6). Results represent mean responses of three to five mice per group. Bars represent SE values.

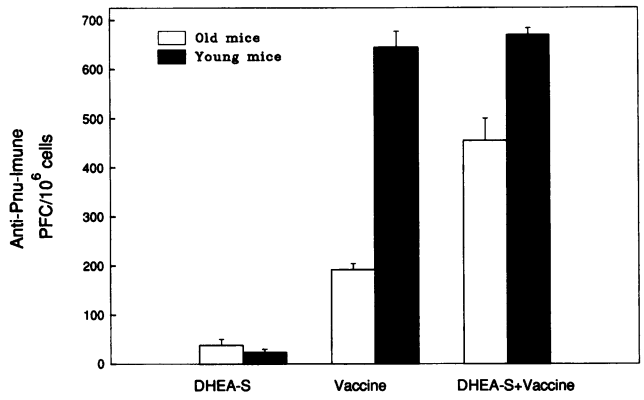

FIG. 4. DHEA-S (100 μg) was administered i.p. into young (4 to 5 months) or old (22 to 24 months) BALB/c mice. Pnu-Imune vaccine was injected i.p. 24 h later into these mice, and the vaccine-specific PFC were measured on day 5. Mean responses of four to six mice are represented, and the bars denote the SE values.

in the DHEA-sulfatase activity. There was no PFC response in the PLN in the mice given vaccine only, whereas mice treated with DHEA and vaccine gave a mild response (18 to 20 PFC per 10^6 cells).

Our previous studies showed that PFC responses to the vaccine from the mucosally associated lymph nodes were not decreased as a function of age and that intraperitoneal (i.p.) but not s.c. challenge is necessary to obtain vaccine PFC response from the MLN (6). To determine the effect of DHEA-S treatment on MLN responses, and to see whether systemic administration has the same effect as local injection, DHEA-S was administered i.p. into old mice which were immunized 24 h later with the vaccine via the same route. This systemic DHEA-S therapy also enhanced the splenic PFC responses from old mice (Fig. 4). As reported earlier, in old mice MLN response to the vaccine was well preserved (6) but it was not enhanced (data not shown). This is in agreement with previous reports that the DHEA-sulfatase activity is higher in spleen than in mucosal tissues such as MLN and Peyer's patches (4).

Since PSs belong to the TI class of antigens, it is unclear as to how DHEA-S therapy was effective in boosting the immune responses of old mice to these antigens. The antibody responses to such type 2 TI antigens as PS are known to require T-cell-derived lymphokines but not an antigen-specific T-helper cell (14). Our laboratory showed that B-cell responses to trinitrophenylated Ficoll, another prototypic type 2 TI antigen, are dependent on supplementation with IL-5 or splenic accessory cells (8, 9), and our unpublished observations suggest that in vitro responses to the Pnu-Imune vaccine also have similar requirements. Therefore, regulation of IL-5 production may not be the primary mechanism since DHEA therapy increases IL-2 secretion while decreasing IL-5 secretion (1, 3, 5). Instead, the immunoenhancing effect of DHEA may depend on its ability to directly affect B-cell function or indirectly enhance IL-1 production by the accessory cells. Another explanation relates to the ability of DHEA to down regulate the age-associated increase in the production of gamma interferon (1), since we and others find that gamma interferon inhibits PS responses in vitro (unpublished observations) and in vivo (21).

Since the efficacy of the Pnu-Imune vaccine in older individuals and in splenectomized patients has not been as high as in young adults, our results with DHEA therapy suggest a means by which the pneumococcal vaccine can be made more effective for such populations.

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