IMMUNE RESPONSE TO INFLUENZA AND PNEUMOCOCCAL VACCINES IN MICE

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Summary. – The immune response of mice injected with influenza vaccine (FluV) or pneumococcal vaccine (PV) given separately or simultaneously was evaluated. Balb/c mice were divided into six groups. Group I served as control, the mice in group II were injected intraperitoneally with PV, in group III intramuscularly with FluV two weeks after the onset of the study. The mice from group IV received PV and 2 weeks later were injected with FluV, mice in group V were given FluV, whereas group VI received both FluV and PV simultaneously. The results showed that the proliferative response of peripheral blood mononuclear cells (PBMC) significantly increased in animals from groups II, V and VI, whereas the proliferation of splenocytes increased in mice from groups II, III, IV, and VI. These observations indicate a comparable effect of both vaccines, at least when the proliferative response of PBMC and splenocytes were considered.

Key words: influenza vaccine; pneumococcal vaccine; proliferative response

Introduction

A considerable number of studies established the role of epidemic and pandemic forms of influenza as a serious cause of morbidity, mortality, and great economic loss. Immunization against the disease has been shown to be highly effective in prevention and minimizing both its outburst and side effects (Williams *et al.*, 2002). Elderly people, splenectomized individuals, and immunocompromised patients under various forms of chemotherapy are prone to severe infections, particularly those caused by *Streptococcus pneumoniae*. These individuals might be protected by vaccination with a protein-polysaccharide conjugate PV (Molrine *et al.*, 2003; van Dijk *et al.*, 2003; Zandvoort *et al.*, 2003). Furthermore, following PV administration, a significant decrease in outburst of invasive pneumococcal diseases among both infants and adults was observed (Black *et al.,* 2002, 2001).

The aim of the present study was to evaluate and to compare the effect of PV and FluV vaccination on a particular immune response in mice. The phagocytic activity of mouse peritoneal cells, the proliferative response of PBMC and splenocytes to Con A, the NK cell cytotoxicity, as well as the PBMC counts before and after vaccination with either FluV, PV, or both of them were assessed.

Materials and Methods

Vaccines. Pneumococcal vaccine (Pneumovax), a 23-valent PV containing 25 mg of the pneumococcal polysaccharide capsules (Danish nomenclature) type 1-5, 6B, 7F, 8, 9N, 10A, 11A, 12F, 14, 15c, 19a, 20, 22f, 23f, and 33f was obtained from Aventis Pasteur. Influenza vaccine, Influvac was purchased from Solvay Pharmaceuticals.

Animals. Two month-old female Balb/c mice (60 animals) were divided into six groups. Group I served as a control. The mice of group II were injected intraperitoneally with 500 µl of PV diluted

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Abbreviations: Con A = concanavalin A; FluV = influenza vaccine; NK = natural killer; PBMC = peripheral blood mononuclear cells; PV = pneumococcal vaccine; WBC = white blood cells

1/25 in saline. The animals from group III were injected intramuscularly two weeks after the onset of the study with 0.1 ml of FluV diluted 1/200 in saline. The mice from group IV received PV at the above-specified doses and were further injected 14 days later with FluV as indicated. The mice from group V were given FluV at the onset of the study, whereas those in group VI were given both FluV and PV simultaneously at the onset of the study. Laboratory examinations were carried out 4 weeks after beginning of the study.

Cells. Blood samples were collected from the tail vein and cell counts were carried out using a Technicon H-2 cell counter (Bayer). PBMC were isolated by Histopaque gradient centrifugation (Sigma) and suspended in complete RPMI-1640 medium containing 1% penicillin, streptomycin, and nystatin, and supplemented with 10% FCS. To obtain splenocytes, the spleens were removed, minced through a fine stainless steel mesh and the cells were suspended in the complete medium. Their viability was over 95%, tested by trypan blue dye-exclusion.

Phagocytosis of latex particles. To collect peritoneal cells, 4.5 ml of endotoxin free physiological saline were injected into the peritoneal cavity of the animals. After 2 mins, 3–4 ml of peritoneal fluid were withdrawn, the cells were sedimented by centrifugation at 250 x g for 10 mins, counted, suspended to concentration of 3 x 10^6/ml complete medium and incubated with 0.1 ml of 5% suspension of 0.8 μ in diameter latex particles (Sigma) for 60 mins at 37°C in an atmosphere containing 5% CO_2. Following incubation, the cells were sedimented by centrifugation, washed twice in PBS pH 7.2, smeared on glass slides and stained using the May-Grünwald-Giemsa method. The latex particles engulfed by the cells were counted under immersion using a light microscope. At least 200 cells from each animal were evaluated.

Proliferative response. 0.1 ml aliquots of PBMC or splenocyte suspension (2 x 10^6/ml of complete medium) were divided into each well of 96 well plates (flat bottom, Greiner, Bio-One) containing 0.1 ml of Con A (10 μg/ml, Sigma). Cultures, set up in triplicates were incubated for 3 days.

One μCi/well of methyl-^{3}H-thymidine (^{3}H-TdR) (5 Ci/mmol, Amersham) was added 18 hrs before harvesting. Radioactivity was measured with an LKB liquid scintillation counter, model 3380.

NK cytotoxicity test. The standard ^{51}Cr-release assay was performed with Yac1 cells – a cell line sensitive to killing effect of murine NK cells serving as target cells (T). 10^6 Yac1 cells suspended in 0.2 ml of complete medium were labeled with 250 μCi of ^{51}Cr (Amersham) for 40 mins at 37°C. Splenocytes or PBMC served as effector cells (E). Final effector-to-target (E:T) ratios for splenocytes varied from 200:1 to 25:1 and for PBMC 100:1. The supernatants from the ^{51}Cr-labeled target cells incubated with effector cells for 4 hrs at 37 °C were collected and the radioactivity was detected with a γ-counter (LKB Instruments). All reactions were carried out in triplicates and the specific ^{51}Cr-release was calculated as described (Hellstrand and Hermodsson 1989).

Statistics. Statistical analysis was carried out using Student's t-test. The results are expressed as a mean ± SEM.

Results

Following administration of the vaccines, the animals behaved as usual and did not show any visible signs of discomfort. Injection of both vaccines caused changes in immune responses. The white blood cell (WBC) counts were significantly higher in the groups: FluV (group III), PV + FluV (group IV) and FluV (group V) compared with the controls (Table 1). Four weeks after the administration of PV (group II) or both PV and FluV (group VI), the WBC counts were similar to that of control animals. The percentage of lymphocytes was significantly lower only in the group of the mice injected with PV and consecutively with FluV (group IV) as compared with the control mice. The amount of hemoglobin and the number of platelets and polymorphonuclear cells did not differ significantly from controls.

A significant decrease in the engulfing activity of peritoneal cells was found 2 weeks after injection of the mice with FluV (group III) compared with the cells from animals in the control group (Fig. 1). Four weeks after administration of PV, FluV, of both PV and FluV or PV followed by FluV (groups II, IV, V, and VI) the percentage of peritoneal cells that engulfed latex particles did not differ significantly from the peritoneal cells of control mice.

The proliferative response of PBMC to Con A was significantly higher in animals 4 weeks after administration of PV, FluV or simultaneous administration of both vaccines (groups II, V, and VI), compared to the control group (Fig. 2). The proliferative response of PBMC in mice given PV

Table 1. Effect of the vaccination with PV and FluV on peripheral blood cell counts

Groups	Hemoglobin (g %)	WBC (x 10^6/ml)	Platelets (x 10^9/ml)	Polymorphonuclear cells (%)	Lymphocytes (%)
I-Control	17.6 ± 0.2	13.9 ± 0.3	1.07 ± 0.02	0.93 ± 0.1	94.3 ± 0.3
II-PV (4 w)	17.8 ± 0.3	13.2 ± 0.6	1.05 ± 0.02	1.11 ± 0.1	93.1 ± 0.4
III-FluV (2 w)	18.0 ± 0.3	16.6 + 0.9*	1.12 ± 0.02	0.84 ± 0.1	93.7 ± 04
IV-PV (4 w) + FluV (2 w)	17.3 ± 0.3	16.6 ± 0.6**	1.05 ± 0.04	0.91 ± 0.1	92.8 ± 0.5*
V-FluV (4 w)	17.8 ± 0.4	16.5 ± 0.9*	1.06 ± 0.02	1.08 ± 0.1	94.5 ± 0.4
VI-PV (4 w) +FluV (4 w)	18.5 ± 0.3	14.3 0± 0.7	1.12 ± 0.03	0.9 ± 0.1	93.0 ± 0.4

* = $p < 0.05$; ** = $p < 0.01$; w = weeks after the administration of the vaccine.

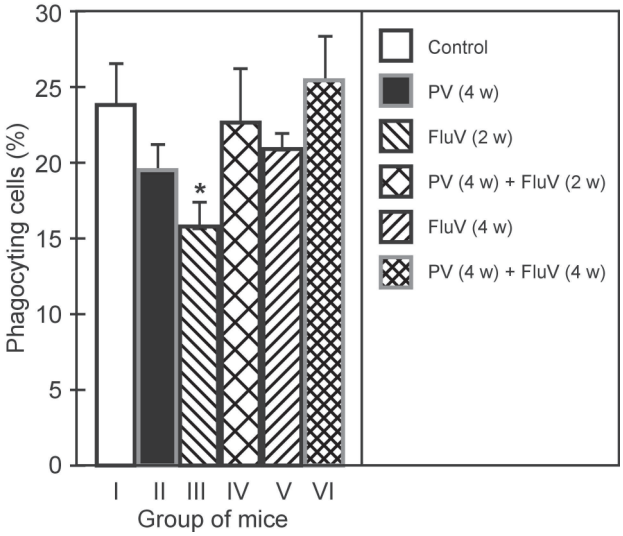

Fig. 1

Phagocytic activity of peritoneal cells following vaccination with PV and FluV

* = p <0.05; w = weeks after the administration of the vaccine.

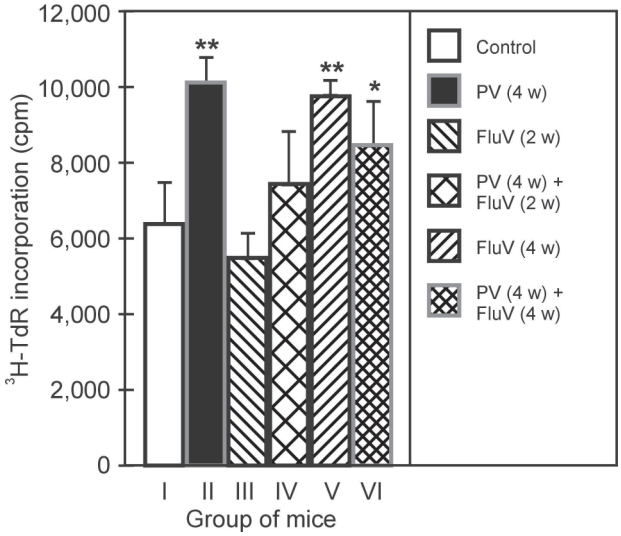

Fig. 2

Proliferative response of PBMC to Con A after vaccination with PV and FluV detected by ^{3}H-TdR incorporation

* = p <0.05; ** = p <0.01; w = weeks after the administration of the vaccine.

followed by injection of FluV (group IV) was higher compared to the group of animals 2 weeks after injection of FluV only (group V), but the values did not differ significantly from the control group.

The proliferative response of splenocytes to Con A was significantly higher 2 weeks after FluV (group III) and 4 weeks after PV administration (group II), and was further increased in mice injected with PV followed by FluV (group IV) in comparison with the controls (Fig. 3). Four weeks after the simultaneous injection of PV and FluV (group VI), the proliferative response of splenocytes to Con A was significantly elevated compared with control mice or those injected with FluV only.

We found no difference in the NK cytotoxicity of the splenocytes and PBMC in the six groups of mice.

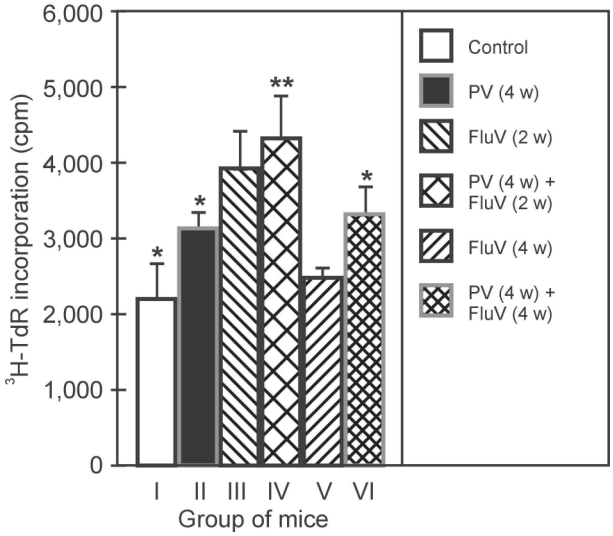

Fig. 3

Proliferative response of splenocytes to Con A after vaccination with PV and FluV detected by ^{3}H-TdR incorporation

* = p <0.05; ** = p <0.01; w = weeks after the administration of the vaccine.

Discussion

The effectiveness of vaccination in prevention of epidemic spread of influenza has been well established (Szucs 2004; Voordouw *et al*., 2003; Centers for Diseases Control and Prevention, 1995). Similarly, the benefit of pneumococcal vaccination has been proven in both children and adults (Sisk *et al*., 2003; Whitney *et al*., 2003). According to Ratcliffe *et al.* (1993) the polymorphonuclear cells provide the first line of defense against the influenza virus. It has been demonstrated by electron microscopy that after incubation of human polymorphonuclear leukocytes

with the influenza virus at 0°C, the viral particles bind to the cell membrane, but they disappear rapidly at 37°C being ingested by cytoplasmic vesicles *via* endocytosis (Yamamoto *et al.*, 2002). Therefore, it is conceivable that certain macrophage functions including their phagocytic activity may be altered. It has been reported that mouse peritoneal granulocytes incubated with antigen obtained from A/Scotland/74 and A/PR-8 influenza viruses show an impaired phagocytic activity (Szydlowska *et al*., 1987; Kowalska *et al*., 1985). Incubation with influenza virus caused a decrease in phagocytic activity of human polymorphonuclear leukocytes (Henricks *et al*., 1985) and monocytes (Gardner and Lawton, 1982). The decrease in the engulfing activity of the peritoneal macrophages following inoculation with FluV observed in the present study is in agreement with these observations.

The proliferative response of the PBMC to Con A after administration of the vaccines differed from that of splenocytes that increased after the inoculation with either PV or FluV with maximal stimulation of the cell proliferation, when both vaccines were given at 2-weeks interval. On the contrary, the proliferative response of PBMC was increased 4 weeks after administration of PV, FluV, or both vaccines simultaneously. This difference in immune response may reflect variation in lymphocyte subsets existing between peripheral blood and spleen (Langeveld *et al*., 2006). NK cytotoxicity did not show any change following injection of PV, FluV, or both vaccines simultaneously. This finding is in agreement with the reports indicating that there is no increase in the mean levels of Influenza A virus-reactive interferon-γ (+) T cells, as well as NK cells following administration of influenza vaccine in adults (He *et al*., 2006).

The results of the present study demonstrate that both vaccines, although assigned to humans, were able to induce comparable immune responses in mice in the examined parameters. Moreover, the proliferative response of the PBMC to Con A was elevated after inoculation with PV, FluV, as well as PV followed by FluV suggesting a synergistic effect of these vaccines. It has been reported that vaccination against pneumococcal infections exerted an additional effect with influenza vaccination in reducing hospitalization for chronic lung diseases, whereas vaccination with influenza vaccine alone did not achieve this effect (Christenson and Lundbergh, 2002). The findings of the present study suggest that vaccination against pneumococcal infections might have a protective effect also against the influenza outbreaks and should be considered as a strategy for immunization against the influenza in humans. Therefore, further studies are warranted to pursuit this concept, taking into consideration the savings that this approach may contribute to public health providers. Indeed, in a previous retrospective survey carried out on 450 individuals vaccinated with either PV, FluV, and with PV followed with FluV every consecutive year, we have observed a decrease in morbidity of flu and flu-related diseases in the group vaccinated with PV only that lasted for 2–3 years (Blay *et al*., 2007).

Summing up, although the experiments have been carried out in mice using human PV and FluV, the animals showed immune response expressed by a decreased phagocytic activity of their peritoneal cells after FluV injection and an increase in the splenocyte and PBMC proliferative response following administration of both vaccines either separately or simultaneously.

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