Indian J Physiol Pharmacol 1999; 43 (4) : 474–478

A SYNERGISTIC DECLINE IN HUMORAL AND CELLULAR IMMUNITY OF DIABETIC MICE ON EXPOSURE TO POLLUTED AIR

S. P. ZAMBAD, S. P. UPGANLAWAR AND S. N. UMATHE*

Department of Pharmaceutical Sciences,
Nagpur University Campus,
Nagpur – 440 010

(Received on November 28, 1998)

Abstract : It is clinically known that diabetic patients are more prone to infectious diseases, due to low immune status. Since, some of the common air pollutants are reported to suppress immune system, how exposure to artificially polluted air influences the immune responses in experimental diabetic mice was studied. A diabetic state was induced by alloxan and mice were exposed to artificially polluted air for 30 days. During the period of exposure, the humoral (antibody titer) and cellular (foot and swelling) immune responses to antigenic challenges with sheep RBC were investigated. The exposure to polluted air produced a significant decline in the immune responses in non-diabetic mice whereas a synergistic decline was observed in diabetic group. Since, daily oral treatment with vitamin E (150mg/kg) significantly prevented the pollution induced immunosuppression, the involvement of free radicals is suggested.

Key words : pollution, immunosuppression, vitamin E, diabetes, free radicals

INTRODUCTION

The common air pollutants such as CO, NO_2, SO_2, etc. are known to produce immunosuppression in experimental animals (1). These pollutants generate free radicals and subsequently induce oxidative stress on biological system (2–4). Free radicals produce cellular injury to the essential components of immune system such as spleen and thymus (5). The diabetic patients are known for a higher susceptibility to infectious diseases (6). They are known to possess handicapped immune system and higher levels of free radicals in their tissues (7–9). If such diabetic subjects are exposed to free radical generating pollutants, then, a futher deterioration immune response can be anticiapted. In view of this, the humoral and cellular immune responses of diabetic mice on exposure to polluted air, have been evaluated in the present study. In order to investigate the involvement of free radicals in pollutant induced immunological alterations, experiments were also carried in vitamin E treated mice.

*Corresponding Author

METHODS

(A) Animals

Swiss mice (National Institute of Nutritrion, Hyderabad), weighing about 25–30g, of either sex were equally divided into groups namely, control (to be exposed to nonpolluted air) and experimental group (to be exposed to polluted air). The mice from control and experimental groups were housed in separate acrylic chambers of same size (60cm×60cm×45cm) having one inlet on each side and two outlets at the top. Mostly, the animals had free access to food (Gold Mohar, Mumbai) in the chamber, except when treatment protocol demanded 18 h of fasting. However, they had free access to water. The temperature was controlled at 25±2°C and light-dark cycle (12-12 h) was maintained.

(B) Grouping

The mice from the control and experimental groups were divided into subgroups namely, Diabetic (DB), Nondiabetic (NDB), Vitamin E treated diabetic (DBE), and Vitamin E treated nondiabetic (NDBE).

(C) Exposure protocol

1) Exposure of control to nonpolluted air :

The normally present air was compressed in metallic gas cylinder. The compressed air was bubbled through distilled water and was allowed to pass in the housing chamber through its inlets continuously for 30 days at a constant rate of perfusion (0.3L/min). The flow rate was controlled with the help of regulator and flowmeter attached to the cylinder.

2) Exposure of experimental group to polluted air :

The smoke emitted by motor bike, at a fixed rate of acceleration; was compressed in metallic gas cylinders fitted with a regulator and flow-water. The outlets of two cylinders containing smoke and normal air respectively, were connected to a common tube through which a mixutre of both compressed gases was allowed to pass to the acrylic chamber through its inlets. The perfusion of chamber with polluted air (0.3L/min.) was round the clock for all 30 days except when the animals were daily taken out for various treatments, blood sampling cleaning of chambers, but at no time for a period exceeding one hour.

(D) Treatment protocol

1) Induction of diabetic state by alloxan :

Three days prior to exposure, the groups DB and DBE received alloxan (150mg/kg, subcutaneously) whereas the groups NDB and NDBE received only 0.9% saline as the vehicle. To confirm the induction of diabetic state in group DB and DBE, the blood glucose levels (BSL) were checked on the 3rd day of alloxan treatment. Only those mice who had BSL more than 200mg/dl were used. On confirmation of diabetic state on 3rd day of alloxan treatment, the mice were subjected to the exposures. As the diabetic condition induced by single dose of alloxan reverses in about 15–20 days, second dose of alloxan was repeated to the DB and DBE

groups after 15 days (i.e., 12th day of exposure). NDB and NDBE groups received the vehicle of alloxan at the same time. All the mice were fasted for 18 h prior to alloxan treatment. The BSL were again tested on 15th and 30th day of exposure to ascertain the diabetic state.

2) Antioxidant therapy :

The group DBE and NDBE daily received vitamin E (150 mg/kg in 6.25% v/v tween 20, orally) from first day to the last day of exposure. Experiments with vehicle (tween 20, 6.25% v/v) were carried out simultaneously.

3) Antigenic challenge :

Mice from all groups were challenged with intraperitonial injection of sheep RBC (0.5×10^9 cells/100g) suspended in 0.9% saline on 14th and 20yth day during exposure. On 27th day, sheep RBC (0.025×10^9 cells) were injected in right paw and 0.9% saline in the left paw.

(E) Immunological studies

1) Humoral immune response (HIR) :

HIR was assessed by estimating antibody titer by heme-agglutination method (10). On 20th and 27th day, blood was withdrawn from retro-orbital plexus of mice, challenged with sheep RBC on 14th and 20th day of exposure. The HIR was determined by titrating serial dilutions of serum with sheep RBC (0.025×10^9 cells) in a microtitrating plate. The maximum number of dilution that produced heme-agglutination was considered as the antibody titer of serum.

3) Cellular immune response (CIR) :

CIR was assessed by foot-pad reaction method (11). The volume of each hind paw was measured by plethysmometer on 27th and 30th day. Increase in paw volume subsequent to the injection of sheep RBC in right paw on the 3rd day, was due to delayed type of hypersensitivity and it was considered as an index of CIR. The volume of left hind paw, in which saline was injected, served as control.

(F) Statistical Analysis :

The data was analyzed by one way ANOVA followed by student-Newman-Keul's test. The difference was considered significant when $P<0.05$.

RESULTS

The humoral immune response (HIR) to sheep RBC has been expressed as mean antibody titer (Table I) and cell mediated immune response (CIR) has been expressed as mean percent rise in paw volume (Table 2). Reslults indicate that HIR and CIR of diabetic mice, even on exposure to normal air, are basically lower ($P<0.05$) than nondiabetic mice. It is further observed that exposure to polluted air significantly reduces the immune response of both, diabetic and nondiabetic mice ($P<0.05$) wherein the decline in responses of diabetic mice is comparatively more than that of nondiabetic mice ($P<0.005$). The studies in vitamin E treated group indicate that such treatment improves the basically low immunological profile of diabetic mice and significantly prevented the pollution

TABLE I : Influence of polluted air on humoral immune response to sheep RBC in diabetic and non diabetic mice.

S.No	Groups	Type of exposure	
		Normal air exposure	*Polluted air exposure*
1.	Non-diabetic	7.6±0.23	5.16±0.18*
2.	Vit. E treated nondiabetic	8.50±0.24	6.16±0.18
3.	Diabetic	6.16±0.33*	4.33±0.23*
4.	Vit. E treated Diabetic	7.23±0.37	5.83±0.18

Values represent the mean of antibody titer (a maximum number of dilution exhibiting heme-agglutination).
± Standard error of mean (n = 10).
* P<0.05, when compared with nondiabetic group exposed to normal air.

TABLE II : Influence of polluted air on cellular immune response (foot-pad reaction) to sheep RBC in diabetic and non diabetic mice.

S.No	Groups	Type of exposure	
		Normal air exposure	*Polluted air exposure*
1.	Non-diabetic	26.28±2.19	15.14±1.5*
2	Vit. E treated nondiabetic	29.66±1.76	21.92±1.94
3	Diabetic	16.15±0.72*	7.59±1.15*
4	Vit. E treated Diabetic	24.28±1.78	19.03±1.14

Values represent the mean percent increase in paw volume.
±Standard error of mean (n = 10).
*P<0.05, when compared with nondiabetic groups exposed to normal air.

induced reduction in HIR and CIR in both diabetic and nondiabetic groups.

DISCUSSION

The observation that experimentally polluted air produced a significant decline in immune responses is in accordance with the earlier reports (1). However, earlier workers, have mostly employed a single pollutant in their studies. Though the mechanism of immunosuppressant effect of pollutants is not very clear, it is suggested that the generation of highly reactive free radicals is the probable cause for the observed immunotoxicity.

It was interesting to note that diabetic mice, even on normal air exposure, exhibited lower immune responses as compared to nondiabetic mice which probably explain the clinically evident higher susceptibility of diabetic patients to infectious diseases (7, 10). The diabetic mice experienced a further decline in their immune responses when they were exposed to polluted air over a period of 30 days. The presence of oxidative stress in diabetic condition and additional

oxidative stress due to polluted air appears to be the probable cause for such immunological responses of diabetic mice. The fact that daily vitamin E treatment could improve low immune status of diabetic mice and also prevented the synergistic decline in immune responses of pollution exposed diabetic mice supports this contention. These observations are also supported by the documented beneficial effect of antioxidant therapy in pollution induced manifestations (5). The observed immunosuppression does not appear to be related to the free radicals directly generated by alloxan (12) as it was only given twice during the period of experimentation. It is more likely related to the subsequent diabetic state as insulin treatment is known to improve the immune status of diabetic patients (13). In conclusion dibetic mice experienced a severe immunodepression on exposure to polluted air and, as vitamin E significantly prevented the same, the involvement of free radical is suggested.

ACKNOWLEDGEMENT

The financial support provided by UGC, New Delhi, in the form of junior research fellowship is acknowledged.

REFERENCES

1. Graham JA, Gardner DE. Immunotoxity of air pollutants. In : Immunotoxicology and Immunopharmacology; Raven Press New York, 1985; 367–380.
2. Elstner EF, Oswald W. Air Pollution : Involvement of oxygen radical. *Free radical Res Comm* 1991; 12(13): 789–807.
3. Menzel DB. Nutritional need in environment intoxication. Vitamin E and air pollution an example. *Environ Health Perspect* 1979; 29: 105–114.
4. Kelly FT, Madway I. The Free radical basis of air pollution focus on ozone. *Respir Med* 1995; 89(10): 647–656.
5. Fujmaki, Hidikaju, Stimiza, Fujio, Effect of acute exposure of NO_2 as PAR. *Arch of Environ Health* 1981; 36: 114–119.
6. Mencacci A, Ruman L, Mosci P, Cenii E. Low dose streptozocin induced diabetes in mice. Susceptibility to infection correlates with the induction of a biased Th_2 like response. *Cell Immunol* 1993; 1: 36–44.
7. Bloomagarden ST. Antioxidant and diabetes. *Diabetic Care* 1997: 670–676.
8. Santini SA, Marra G, Giardina B, Cotroneo P, Mordente GE, Manto E, Ghirlanda G. Defective plasma antioxidant defences and enhanced susceptibility to lipid peroxidation in uncomplicated IDDM. *Diabetes* 1997; 46(11): 1853–158.
9. Peterson ML, Rommon N, House D, Harder S. *In vitro* responsiveness of lymphocytes of phytohaemoglutination. *Arch of Environ Health* 1978; 33(2): 59–63.
10. Miller LE, Ludke HR, Peacock TE, Tomer RH. In : Manual of Immunology. Second edition. Leg and Febiger, London 1991; 1–57.
11. Papadimitriou C, Hann H, Naher H, Kaufmann SH. Celleular immune response to sheep erythrocytes: Interrelationship between prolifiration of popliteal lymph node cells and foot pad swelling. *Immunobiology* 1983, 1964: 361–369.
12. Koichi Sakurai, Taketo Ogiso Generation of alloxan radical in rat islet cells: purification of NADPH:cytochrome p–450 reductase. *Bio Pharm Bull* 1994; 14(11) : 1451–1455.
13. Gaulton GN, Schwartz JL, Eordely DD. Assessment of diabetogenic drug alloxan and streptozocin as models for the study of immune defect in diabetic mice. *Diabetologia* 1985; 28(10): 769–775).