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Effect of amphotericin B lipid formulation on immune response in aspergillosis

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Abstract

The immune response against *Aspergillus fumigatus* has been studied during infection and therapy in order to understand the mechanism of pathogenesis and the effect of treatment with amphotericin B. With this in view an animal model of aspergillosis was developed in Balb/c mice by intravenous injection of an optimized dose of 3.6×10^6 *A. fumigatus* spores. Infection due to *Aspergillus* was well established by histopathological examination and fungal load in the animal. Lesions and eosinophil infiltration was observed in the infected tissues which indicated the involvement of a Type I hypersensitivity response. Evaluation of serological parameters indicated high levels of interleukin-4 (IL-4) and *A. fumigatus* specific IgG antibodies. The reduction in fungal load and modulation of immune response in the infected mice was studied following treatment with amphotericin B/cholesterol hemisuccinate vesicles (ABCV). The results clearly indicated significant reduction in the fungal load, disappearance of eosinophils and lesions with the appearance of macrophages and neutrophils in the infected lung tissue, a decrease in IL-4 (fourfold) and a concomitant increase of interferon-γ (IFN-γ; twofold) with an improvement in general condition of mice. In the non-treated mice, the rise of IL-4 level indicated the association of T_H2 cell response with susceptibility to infection while the increase of IFN-γ in the treated group suggested that T_H1 cell response may be involved in resistance to *Aspergillus* infection.

Keywords: *Aspergillus fumigatus*; Immuneregulation; Amphotericin B; Sterol vesicles; Cytokines; Lesions

1. Introduction

Aspergillosis, a fungal disease caused by *Aspergillus fumigatus* comprises two major clinical forms, allergic and invasive (Rinaldi 1983; Bodey and Vartivarian 1989). Invasive aspergillosis remains a very important cause of fungal infections in immunosuppressed hosts especially organ transplant cases with mortality approaching 100% (Kusne et al., 1992; McWhinney et al., 1993; Walsh and Pizzo, 1994). The threat of allergic aspergillosis has also been realized in the last few years (Rosenberg et al., 1997). The factors involved in the pathogenesis of *A. fumigatus* infec-

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tion are not well understood even though there have been many studies carried out on different animal models of aspergillosis.

An allergic bronchopulmonary aspergillosis model was developed by Kurup et al. (1992) by intraperitoneal and intranasal exposure of Balb/c mice to particulate *A. fumigatus* antigen. They observed blood and lung eosinophilia and increase in the levels of IgG_1 and IgE antibodies (Kurup et al., 1994, 1997). Invasive disease which is characterized by hyphal invasion and destruction of pulmonary tissue is often fatal. An invasive model for aspergillosis was developed by Nagai et al. (1995) using immunosuppresants before and after intravenous administration of *A. fumigatus* spores.

In the present study, an optimized dose of 3.6×10^6 *A. fumigatus* spores without any immunosuppresants was administered intravenously for developing infection which was confirmed by histopathological and immunological parameters. A lipid formulation, amphotericin B cholesterol hemisuccinate vesicles (ABCV) which was found to be significantly less toxic than free amphotericin B (amphotericin B deoxycholate; AmB_{DOC}) was developed and used for treating the infected mice (Saxena et al., 1998). Therefore the current work is aimed at elucidating the role played by various factors involved in suppressing or promoting this infection. Humoral response and cytokine profile in infected, ABCV treated and control groups of mice were examined.

Many workers have investigated cytokine production by CD_4T_H cells in order to determine the pattern of susceptibility and resistance to fungal infections (Romani et al., 1992; Cenci et al., 1995, 1997). They reported that resistant mice produced interferon-γ (IFN-γ) whereas in susceptible mice, interleukin-4 (IL-4) production was observed. Other workers have also reported an increase in survival of infected mice by external administration of IFN-γ and IL-4 receptor which suggested that IL-4 and IFN-γ are involved in susceptibility and resistance to *A. fumigatus* respectively (Romani et al., 1991; Puccetti et al., 1994; Nagai et al., 1995; Cenci et al., 1997).

We detected *A. fumigatus* specific antibodies and observed T_H1–T_H2 cross-regulation in immune response against *A. fumigatus*. It was observed that non-treated mice produced T_H2 cytokines whereas ABCV-treated mice which showed improved survival produced T_H1 cytokines. Therefore it seems that activation of T_H1 or T_H2 cytokines is an important factor in disease progression (Clerici and Shearer, 1993).

2. Materials and methods

2.1. Animals

Male Balb/c mice (20 g body wt) were obtained from the laboratory animal facility of National Institute of Nutrition, India and main-

Table 1
Plan of infection and treatment for immunological studies

Description of group	Schedule of saline/spore injection	Schedule of treatment
Normal control	Mice not injected	No treatment done
Drug control	Injected with physiological saline by i.v. route	Intravenously administered with 12 mg/kg wt ABCV 24 h after injection of saline
Non-treated	Intravenously administered with 3.6×10^6 spores of *A. fumigatus*	Intravenously administered with Tris–HCl buffer 24 h after injection of spores
Treated	Intravenously administered with 3.6×10^6 spores of *A. fumigatus*	Intravenously administered with 12 mg/kg wt ABCV 24 h after injection of spores

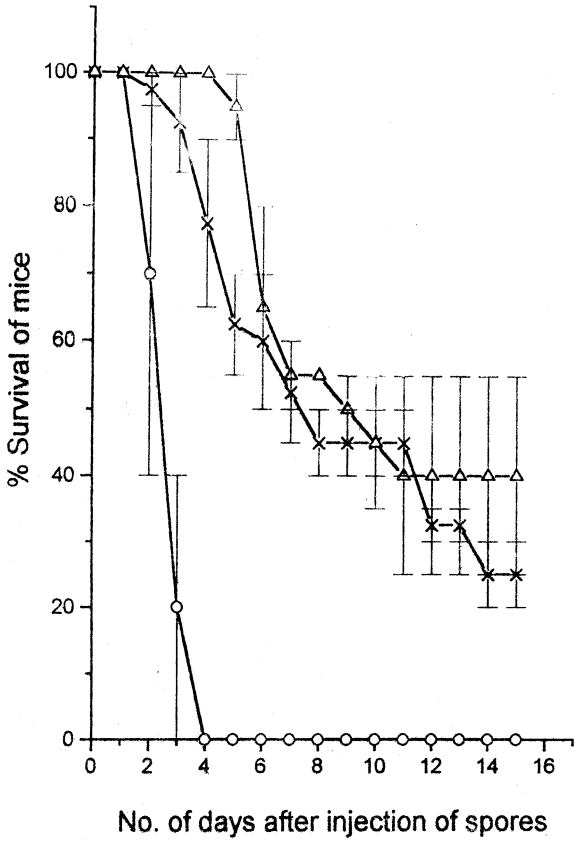

Fig. 1. Effect of intravenous administration of *A. fumigatus* spores on the % survival of Balb/c mice. Each infected group consisted of 20 mice. △, 1.8×10^6 spore injected mice; ×, 3.6×10^6 spore injected mice, ○, 1.8×10^7 spore injected mice. Values are expressed as mean of % survival in two experiments ± S.E.M.

tained in the animal house of University of Delhi South Campus.

2.2. Microorganism used for infection

A. fumigatus, a pathogenic strain isolated from an invasive patient was a kind gift from Dr N.A. Kshirsagar of KEM Hospital, Mumbai.

2.3. Drugs, lipids and general chemicals

Amphotericin B and cholesterol hemisuccinate were obtained from Sigma (St Louis, MO). Cytokine detection kits were obtained from Endogen, USA. All other chemicals used in the study were of analytical grade.

2.4. Development of a murine model for aspergillosis

The fungus was cultured on Sabouraud Dextrose agar medium for 48 h. The spores were suspended in normal sterile saline and counted in a hemocytometer. Groups of animals were injected with 0.25 ml of 0.15 M saline containing spores varying from 1.8×10^6 to 1.8×10^7 for development of *Aspergillus* infection.

2.5. Preparation of ABCV

ABCV employed for the treatment of infected mice was prepared as described earlier (Saxena et al., 1998). In brief, amphotericin B and cholesterol hemisuccinate were cosolubilised in methanol and the resulting dry film was hydrated with Tris–HCl buffer to obtain ABCV.

2.5.1. Comparison of toxicity of ABCV and free amphotericin B

Toxicity due to administration of free amphotericin B (amphotericin B deoxycholate; AmB_{DOC}) is well documented in the literature. In an attempt to reduce the toxicity of amphotericin B, ABCV was developed and its acute toxicity, in vitro toxicity and nephrotoxicity were compared with AmB_{DOC}. Acute toxicity was evaluated by intravenously administering the drug and observing any mortality within 2 h. To determine the maximum tolerated dose, survival was checked for a period of 4 days. The different doses chosen for the evaluation of nephrotoxicity and in vitro toxicity of ABCV and AmB_{DOC} were based on the maximum tolerated dose of the formulation. In vitro toxicity of ABCV to erythrocytes was determined as described earlier (Saxena et al., 1998). In brief, RBCs were drawn from Balb/c mice, incubated with ABCV or AmB_{DOC} at 37°C for 1 h and hemoglobin released was measured. Similarly nephrotoxicity was evaluated by measuring serum creatinine levels of mice injected with ABCV or AmB_{DOC}.

2.6. Schedule of infection and drug administration to various groups of mice

We used 3.6×10^6 spores of *A. fumigatus* to develop infection in Balb/c mice as 25% mice survived for 15 days giving sufficient time for study of an immune response. Based on observations of the condition of the mice, their survival and fungal load, 12 mg/kg wt ABCV was used as a model treatment for elucidating the immune profile during cure of infection. Immune responses were examined in the infected mice with and without the administration of drug with appropriate controls as described in Table 1.

2.7. Study of various parameters to determine the therapeutic efficacy of ABCV

The efficacy of the drug was evaluated on the basis of

1. the general condition of mice;
2. survival;
3. fungal load, and
4. the histopathology of their lung tissue.

General condition and survival of mice was checked everyday for 15 days. Fungal load in various organs of infected mice was determined as described earlier (Saxena et al., 1998).

2.8. Histopathology of the lung tissue at ultrastructural level

Histopathology of the infected tissues was studied by the use of electron microscopy.

2.8.1. Scanning electron microscopy

Mice were sacrificed, lung tissue excised and washed thoroughly in normal saline. It was sliced into small segments and fixed for 4–16 h in 2.5% glutaraldehyde buffered with 0.1 M sodium phosphate buffer pH 7.2. After fixation, the tissue samples were washed in sodium phosphate buffer and post fixed in 1% osmium tetroxide for 2 h at 4°C. Following fixation, the specimens were dehydrated in a series of acetone on ice, critical point dried using liquid CO_2, coated with gold and observed in a Philips SEM 505 electron microscope at 20 kV.

2.8.2. Transmission electron microscopy

The lung tissue samples were fixed and post fixed as described above. Specimens were buffer washed, dehydrated in acetone and embedded in TAAB resin (TAAB Labs, Reading, UK). Ultrathin sections (60–70 nm) were cut using a Reichert Ultracut E microtome, mounted on

Table 2
Fungal load (CFU/organ) in *A. fumigatus* infected Balb/c mice with and without treatment with amphotericin B[a]

Treatment	Dosage (mg/kg)	Colony counts [$\log_{10}$ (mean CFU $\pm$ S.E.)]			
		Kidney	Liver	Spleen	Lung
Non-treated	–	4.01 ± 3.02	3.74 ± 3.04	3.10 ± 2.17	2.54 ± 1.67
AmB_{DOC}	1	3.73 ± 2.76*	3.45 ± 2.99	3.15 ± 2.11	2.03 ± 1.07**
ABCV	2	3.14 ± 2.23**	3.15 ± 2.58**	2.85 ± 1.92**	1.79 ± 1.33**
ABCV	4	3.14 ± 2.10**	2.39 ± 1.76**	2.87 ± 1.94**	1.55 ± 0**
ABCV	8	2.99 ± 2.34**	2.36 ± 1.21**	2.07 ± 0.92**	1.55 ± 0**
ABCV	12	U.D.	2.10 ± 1.52**	U.D.	U.D.

[a] CFU, colony forming units; U.D., undetectable. The CFU was determined in each group of mice after 5 days of injection of 3.6×10^6 spores. Values are expressed as $\log_{10}$ mean CFU of two readings of two animals in each group $\pm$ S.E.M. Analysis of variance between the non-treated and treated values was heterogeneous.

* t-values were significant at 5% level within the respective organ.

** t-values were significant at 1% level within the respective organ.

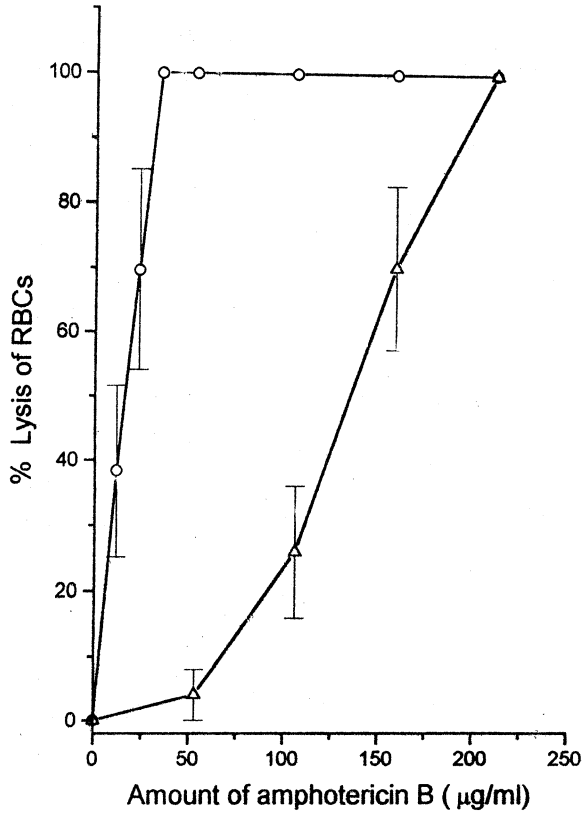

Fig. 2. Erythrocyte lysis caused by amphotericin B when delivered through AmB_{DOC} and ABCV. Values are expressed as mean of % lysis of two experiments ± S.E.M. ○, AmB_{DOC}; △, ABCV.

uncoated 300 mesh copper grids and double stained with uranyl acetate (10 min) and lead citrate (10 min). Sections were examined using a Philips CM10 transmission electron microscope.

2.9. Indirect ELISA

Indirect ELISA was carried out as described by Banerjee et al. (1995) with some modifications. In brief, antigen-coated plates were incubated with 1:25 diluted serum for 3 h at 37°C. Protein A peroxidase was then added for detection of IgG antibodies by use of substrate *O*-phenylenediamine. Colour developed was measured against blank at 490 nm in a NUNC ELISA reader.

2.10. Cytokine analysis

Serum levels of IL-4, IFN-γ, interleukin-2 (IL-2) and tumor necrosis factor-α (TNF-α) were determined by ELISA method as given in the protocols provided with the kits. In brief, antimouse cytokine precoated plate was incubated with plate reagent, standards and samples for 2 h at 37°C. After washing, antimouse cytokine peroxidase was added and incubated for 1 h at 37°C. After a second washing tetramethylbenzidine (TMB) substrate was added and kept in the dark for 30 min after which reaction was stopped with H_2SO_4. The absorbance of the plate was read at 450 nm. A standard graph was made for each cytokine and the serum values for different groups of mice were estimated from the standard graph.

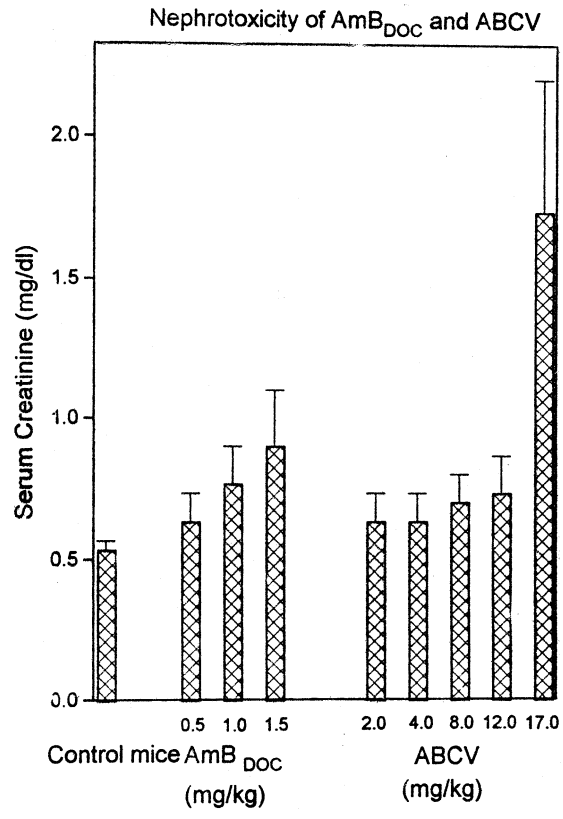

Fig. 3. Changes in serum creatinine in Balb/c mice treated with AmB_{DOC} or ABCV. Each treatment group consisted of four mice. Values are expressed as mean of serum creatinine (mg/dl) of two experiments ± S.E.M.

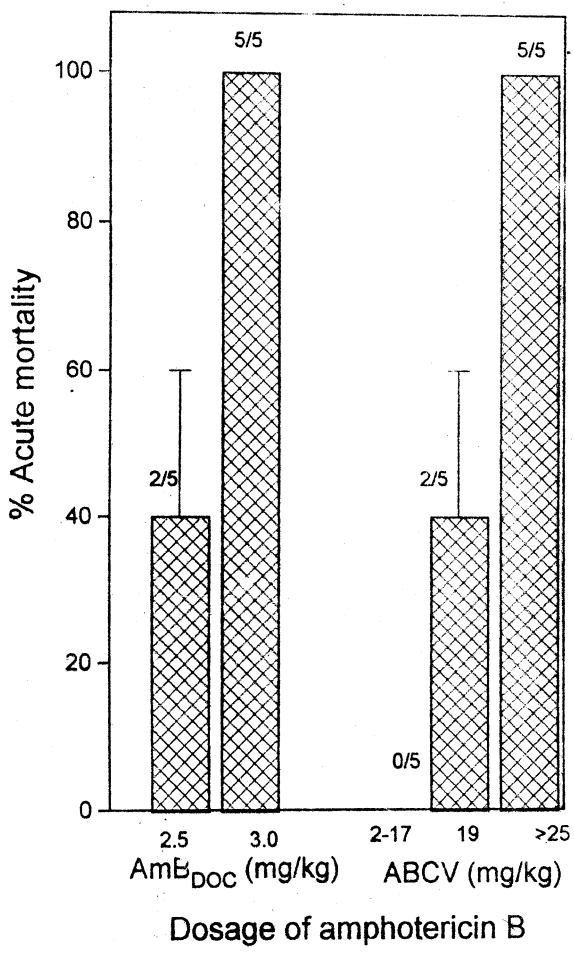

Fig. 4. Acute mortality of mice treated with AmB_{DOC} or ABCV. Numbers at the top of each bar indicate the number of deaths per number of mice injected within 2 h of administration of drug. Each treatment group consisted of five mice. Values are expressed as mean of two experiments ± S.E.M.

For each sample, duplicates were run and the mean values were expressed for individual cytokines.

2.11. Statistical analysis of colony forming units and survival

The colony forming units data were statistically analyzed by applying the Student's t-test to check for heterogeneity between non-treated and treated means. The survival data was analyzed using the χ^2-test on a 2×2 table to find out the heterogeneity between non-treated and treated survival ratios on each day. Sera from four mice were assayed in duplicates on each day for antibody or cytokine levels. Values were expressed as the mean of two readings of two experiments ± S.E.M.

3. Results

3.1. Animal model for aspergillosis

The survival of mice was dependent on the dose

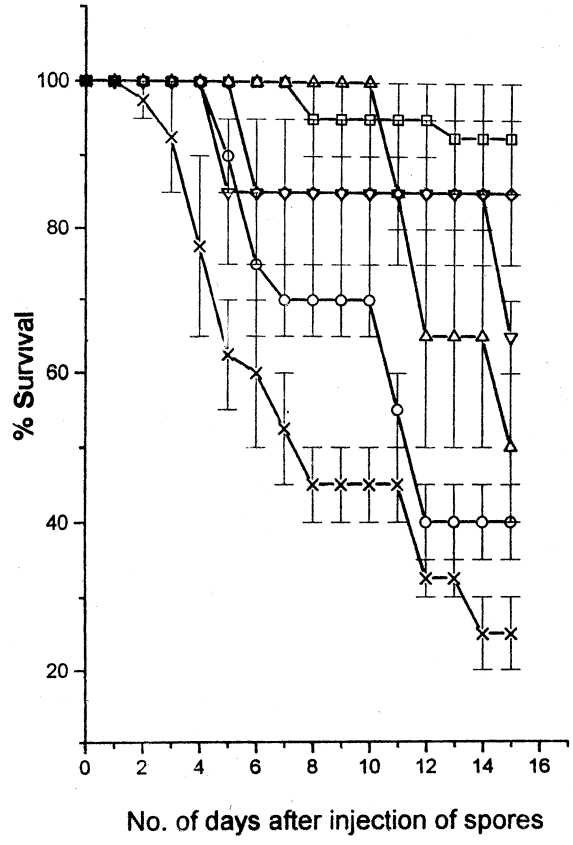

Fig. 5. Effect of different doses of ABCV on survival of *A. fumigatus* infected Balb/c mice. Amphotericin B treatment was given 24 h after injection of 3.6×10^6 spores. ×, infected mice; △, infected mice treated with 2 mg/kg wt ABCV; ▽, 4 mg/kg wt ABCV; ◇, 8 mg/kg wt ABCV; □, 12 mg/kg wt ABCV; ○, 1 mg/kg wt AmB_{DOC}. Each treatment group consisted of 20 mice. Values are expressed as mean of % survival of two experiments ± S.E.M.

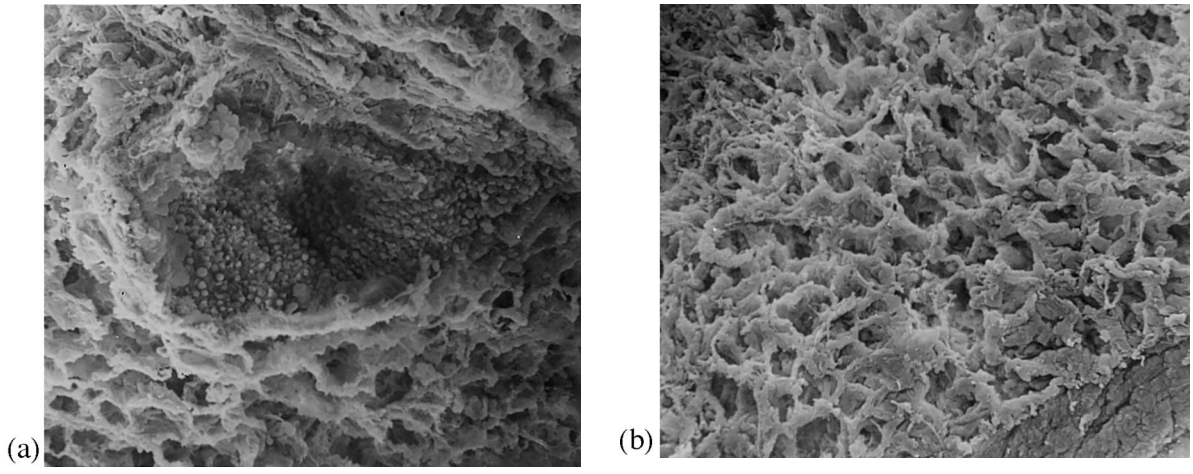

Fig. 6. Scanning electron micrographs of lung tissue of Balb/c mice on the 5th day. (a) In lung tissue of infected mice typical lesions were observed in which fungal spores colonize and invade tissues, × 353. (b) Treated mice did not show lesions in the lung tissue which is suggestive of control of infection, × 353.

of spores injected (Fig. 1). A spore dose of 1.8×10^7 resulted in acute infection with high mortality. On the 2nd and 3rd day, variation in the percent survival was observed between the two separately carried out experiments but all the mice died by the 4th day. On other hand, when 3.6×10^6 spores were administered, 25% of the infected animals survived even till the 15th day. The optimized dose of 3.6×10^6 spores was used for study of immune response to *A. fumigatus*. *Aspergillus* infection in the mice was well established by the presence of eosinophils and lesions in infected tissue with an increase in *A. fumigatus* specific antibodies. Table 2 shows the colony forming units in different organs which reflect the dissemination of this infection.

3.2. Toxicity of ABCV to Balb/c mice

Figs. 2–4 show the effect of free amphotericin B and ABCV on erythrocyte lysis, nephrotoxicity and acute toxicity in Balb/c mice respectively. ABCV was about six times less toxic than AmB_{DOC} to mouse erythrocytes. Nephrotoxicity was also significantly reduced when amphotericin B was delivered using cholesterol hemisuccinate vesicles. Mice receiving 12 mg/kg wt ABCV had lower serum creatinine levels in comparison to mice administered with 1.5 mg/kg wt AmB_{DOC}. The maximum tolerated dose was observed to be 2 mg/kg wt via intravenous route for AmB_{DOC} and it increased to 17 mg/kg wt when cholesterol hemisuccinate vesicles were used for delivery of amphotericin B. As we have reported earlier, the reduction in toxicity of amphotericin B when delivered by way of cholesterol hemisuccinate vesicles is due to altered biodistribution (Wang et al., 1995; Saxena and Ghosh, 1998).

3.3. Survival, fungal load and general condition of infected and treated mice

Mice infected with *A. fumigatus* showed loss of gait and suffered convulsions, from the 2nd to 3rd day and by the 5–6th day, 50% of the animals died. However, the remaining mice showed an improvement in their general condition and survived until the 16th day. Mice treated with 12 mg/kg wt ABCV showed a general loss of gait and mild convulsions but 92.5% of these animals survived (Fig. 5). Moreover, mice in this group recovered earlier and by the 6th day, they were observed to be normal. Table 2 indicates colony forming units isolated from various tissues of treated and non-treated mice. Fungal load in in

fected mice was observed to be reduced by 99% with 12 mg/kg wt ABCV treatment. When treated with 2, 4 and 8 mg/kg wt ABCV, the decrease in the fungal load was observed to be 79, 86 and 92% respectively. Around 43% decrease in fungal load was observed when treatment was done with 1 mg/kg wt AmB_{DOC}. Since maximum therapeutic efficacy was achieved at 12 mg/kg wt, we chose this as the model treatment for all subsequent histopathological and immunological experiments. The χ^2-test reveals that there was heterogeneity in survival ratios between non-treated and ABCV-treated mice from the 4th day as indicated by the χ^2-value (5%) whereas the AmB_{DOC}-treated group did not show a significant difference at χ^2 (5%).

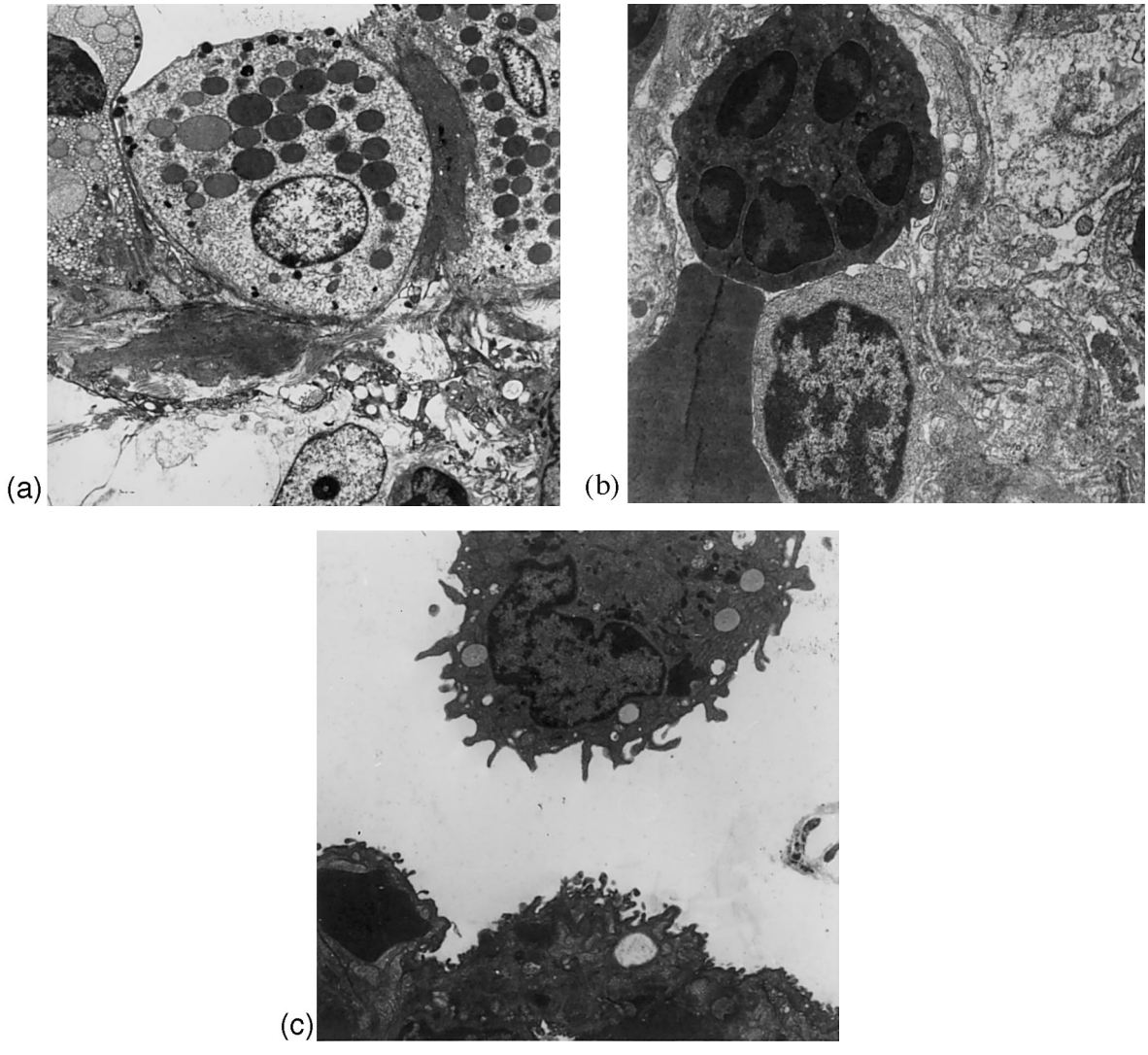

Fig. 7. Transmission electron micrographs of lung tissue of Balb/c mice on the 5th day. (a) In infected mice eosinophilia was observed which is indicative of pathogenic Type I hypersensitivity response, $\times 4814$. (b) In treated mice infiltration of neutrophils, $\times 9628$ and (c) macrophages were observed indicating T_H1 cellular response which is reported to be involved in antifungal activity, $\times 6806$.

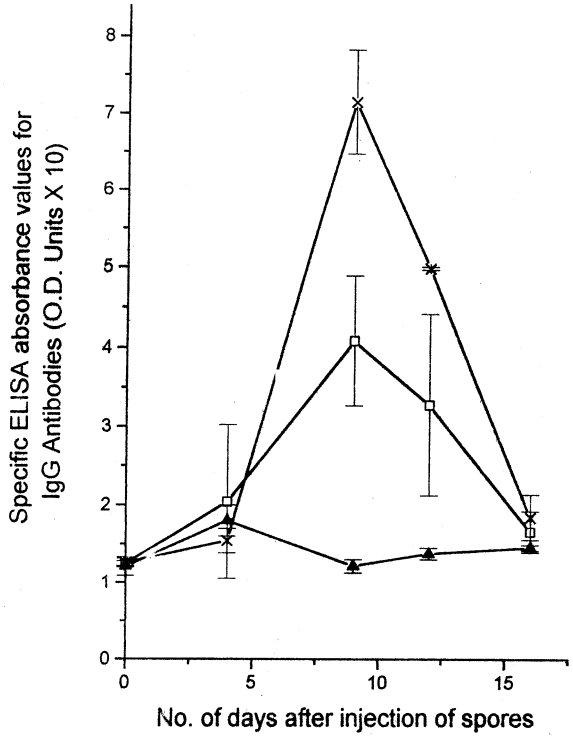

Fig. 8. *A. fumigatus* specific IgG antibodies levels in infected and treated mice. ABCV treatment was 24 h after injection of 3.6×10^6 spores. ▲, normal mice; ×, infected mice; □, infected mice treated with 12 mg/kg wt ABCV. Four mice were sacrificed from each group on various days and serum obtained and evaluated for antibody levels in duplicate. Values are expressed as mean of optical density (O.D.) readings of two experiments ± S.E.M.

3.4. Histopathology of lung tissue of infected and treated mice

Scanning electron micrographs confirm the utility of the infection model as lesions were demonstrated in lung tissue of mice infected with 3.6×10^6 *A. fumigatus* spores. Such lesions were not detected in normal and treated mice which confirms that treatment with ABCV circumvented the spread of fungus (Fig. 6). Transmission electron microscopy of infected lung tissue shows infiltration of eosinophils suggesting a Type I hypersensitivity response due to severe infection of *Aspergillus* (Fig. 7a). Fig. 7b,c shows a reduction in eosinophilia in the treated group of mice and the host response to the fungus in the form of neutrophils and macrophages was observed.

3.5. Level of A. fumigatus specific antibodies in infected and treated mice

The specific antibodies against diagnostically relevant antigens of *A. fumigatus* were not detected before the 9th day after spore challenge. However specific IgG antibodies were detected on the 9th and 12th days by ELISA. A sixfold increase in antibody level in non-treated mice (on the 9th day) as compared to around threefold increase in treated mice (Fig. 8) was noticed. A heavy fungal load (Table 2) in non-treated mice corresponds well with increased level of antibodies.

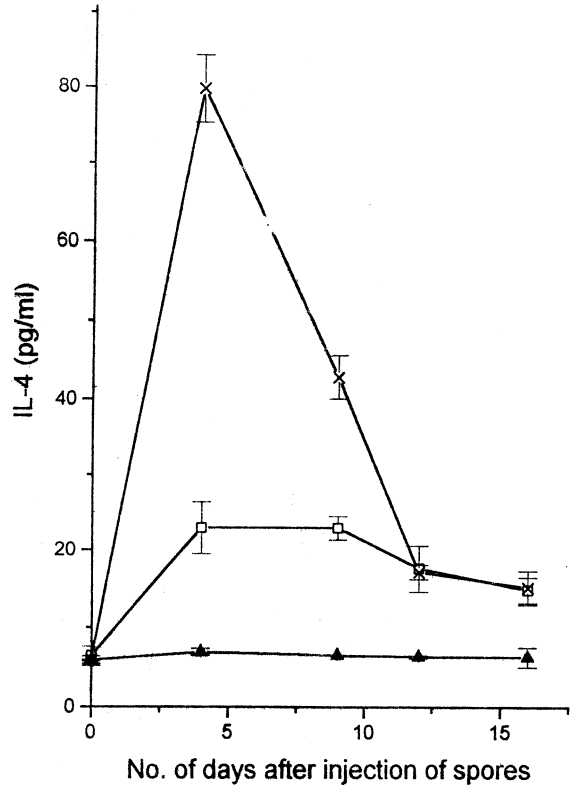

Fig. 9. IL-4 levels in infected and treated mice. ABCV treatment was 24 h after injection of 3.6×10^6 spores. ▲, normal mice; ×, infected mice; □, infected mice treated with 12 mg/kg wt ABCV. Four mice were sacrificed from each group on various days and serum obtained and evaluated for IL-4 levels in duplicate. Values are expressed as mean of two experiments ± S.E.M.

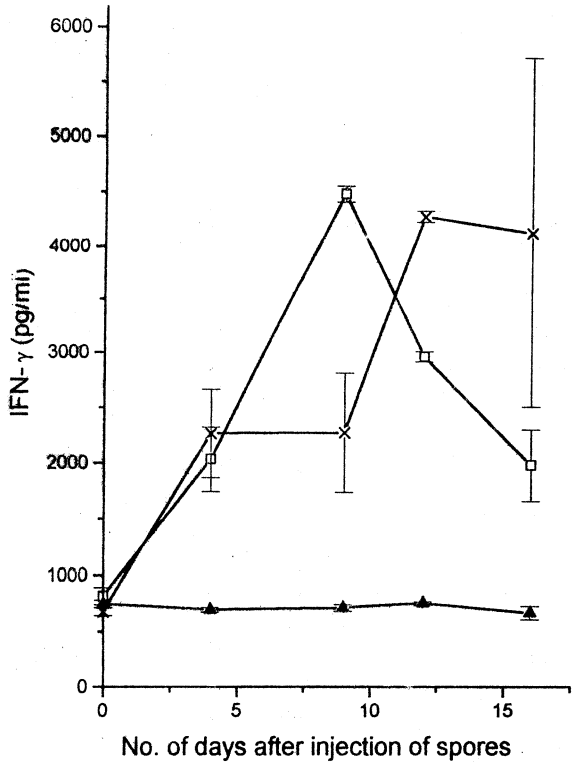

Fig. 10. IFN-γ levels in infected and treated mice. ABCV treatment was 24 h after injection of 3.6×10^6 spores. ▲, normal mice; ×, infected mice; □, infected mice treated with 12 mg/kg wt ABCV. Four mice were sacrificed from each group on various days and serum obtained and evaluated for IFN-γ levels in duplicate. Values are expressed as mean of two experiments ± S.E.M.

3.6. Elucidation of T_H cell subsets and cytokine profile

In order to understand the T_H cell subset associated with cure of infection, cytokine secretion patterns were studied on various days. Normal controls, drug injected, infected and treated mice were sacrificed on various days to determine the serum levels of IL-2, IL-4, IFN-γ and TNF-α. It was observed that antibody and cytokine levels were almost similar in normal control and drug control groups.

Fig. 9 shows that IL-4 was maximum on day 4 in non-treated mice and by day 12 its levels decreased sharply with a corresponding rise of IFN-γ. That is, an initial T_H2 response in non-treated mice is later changed to a T_H1 response. On the other hand, IFN-γ reaches its peak value on day 9 in treated mice (Fig. 10).

This murine model displays a well balanced T_H1–T_H2 cross-regulation. In the non-treated mice, we observed initial high levels of IL-4 with low IFN-γ levels but later on, the infection levels of IFN-γ rose with a concomitant decrease in IL-4 levels. Similarly in the treated group of mice, the IFN-γ reaches peak values earlier with corresponding low levels of IL-4. The levels of IL-2 were detected to be higher in non-treated mice especially on day 4. No significant rise in IL-2 levels was observed in treated mice (Fig. 11).

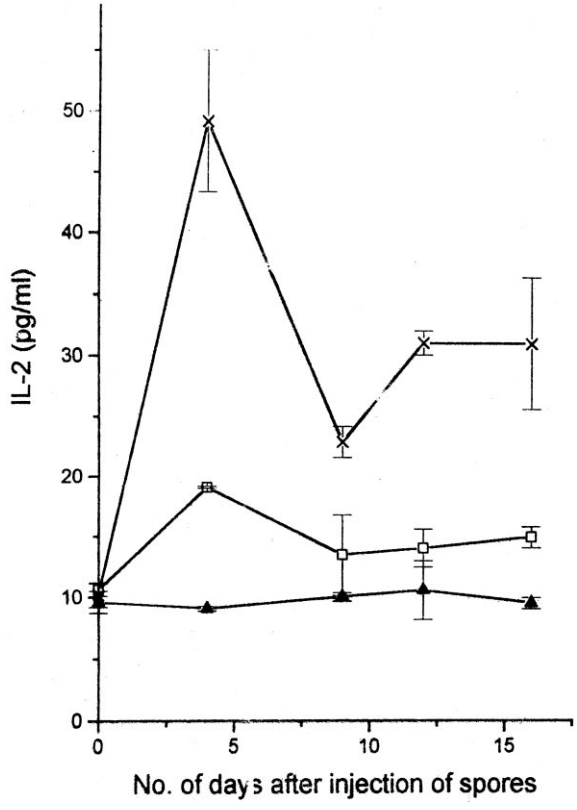

Fig. 11. IL-2 levels in infected and treated mice. ABCV treatment was 24 h after injection of 3.6×10^6 spores. ▲, normal mice; ×, infected mice; □, infected mice treated with 12 mg/kg wt ABCV. Four mice were sacrificed from each group on various days and serum obtained and evaluated for IL-2 levels in duplicate. Values are expressed as mean of two experiments ± S.E.M.

4. Discussion

Many investigators have demonstrated in vivo and in vitro that T_H1 cytokines are associated with resistance to fungal infections (Rex et al., 1991; Roilides et al., 1993; Nagai et al., 1995) while others observed that T_H2 cytokines are produced in susceptible mice (Romani et al., 1992; Cenci et al., 1997). However, not many studies have been carried out on the immune response during treatment of infection which will lead to a better understanding of the factors involved in the treatment process. In the present communication, we have used ABCV for treating the infected mice and the immune response was evaluated during infection and therapy in order to understand the role played by various factors in suppressing or promoting this fungal infection.

We have also observed that the T_H2 cell response is associated with susceptibility during aspergillosis. In the non-treated mice which showed heavy fungal load, low survival and lesions in the infected tissue, a T_H2 response with the presence of eosinophils was observed. The presence of eosinophilia in non-treated mice indicates Type I hypersensitivity reaction to *Aspergillus* antigen. It has been reported in the literature that IL-4 causes an increase in IgE levels leading to mast cell degranulation and release of mediators which serves to attract a large number of eosinophils to the site. Eosinophil activation leads to the release of a number of inflammatory mediators leading to excessive tissue damage (Kuby, 1994). Therefore in the current study, increased levels of IL-4 and eosinophils observed in the non-treated mice correlate well with high mortality. Other investigators have also reported lung eosinophilia during *A. fumigatus* infection in mice (Kurup et al., 1992) and humans (Rosenberg et al., 1997).

Association of T_H1 cellular response with resistance to *A. fumigatus* as reported by others also seems to be true in the present study (Cenci et al., 1997). In the treated mice, a rise in IFN-γ levels coincides with improvement in the condition of mice (around the 6th day). Moreover, it was observed that eosinophilia and IL-4 levels were reduced significantly indicating the inhibition of T_H2 response and there was heavy infiltration of macrophages and neutrophils. It is well documented in literature that IFN-γ brings about its antifungal activity through activation of cells such as macrophages and neutrophils (Kuby, 1994).

In the present study it was also observed that there was enhanced levels of TNF-α in treated mice (unpublished data). It has been reported that activation of macrophages by IFN-γ promotes TNF-α production (Kuby, 1994). Therefore it appears that activation of macrophages and neutrophils by IFN-γ and TNF-α respectively in combination with amphotericin B bring about effective fungicidal action.

Cases of *Aspergillus* species causing invasive, life threatening infections in immunocompromised hosts such as those suffering from AIDS and undergoing organ transplants continue to be reported (McWhinney et al., 1993; Morrison et al., 1994). Documented invasive aspergillosis has a high mortality rate surpassing 60% even with antifungal therapy (Rinaldi, 1983). Therefore there is a need for safer and effective therapeutic measures. This understanding of the immune response during infection and treatment will pave the way for combined therapy wherein the treatment is based not only on antifungal property but also on modulation of immune response for better management of disease.

In the current report an infectious model for aspergillosis was developed and characterized by histopathological and immunological parameters. The non-treated mice showed high mortality with heavy fungal load, poor general condition, lesions in infected tissue, high levels of *A. fumigatus* antibodies and T_H2 cellular responses. Treated mice showing T_H1 response were able to circumvent the infection and showed an improved survival rate.

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References

Banerjee, B., Madan, T., Sharma, G.L., Prasad, H.K., Nath, I., Sarma, P.U., 1995. Characterization of glycoprotein antigen (45KD) of *Aspergillus fumigates*. Serodiagn. Immunother. Infect. Dis. 7, 147–152.

Bodey, G.P., Vartivarian, S., 1989. Aspergillosis. Eur. J. Clin. Microbiol. Infect. Dis. 8, 413–437.

Cenci, E., Mencacci, A., Spaccapelo, R., Tonnetti, L., Mosci, P., Enssle, K.H., Puccetti, P., Romani, L., Bistoni, F., 1995. T helper cell type 1 (Th1)- and (Th2)- like responses are present in mice with gastric candidiasis but protective immunity is associated with Th1 development. J. Infect. Dis. 171, 1279–1288.

Cenci, E., Perito, S., Enssle, K.H., Mosci, P., Latge, J.P., Romani, L., Bistoni, F., 1997. T_H1 and T_H2 cytokines in mice with invasive aspergillosis. Infect. Immun. 65, 564–570.

Clerici, M., Shearer, G.M., 1993. A $T_H1 \rightarrow T_H2$ switch is a critical step in the etiology of HIV infection. Immunol. Today 14, 107–111.

Kuby, J., 1994. Immunology, 2nd edn. W.H. Freeman, San Francisco, CA.

Kurup, V.P., Mauze, S., Choi, H., Seymour, B.W.P., Coffman, R.L., 1992. A murine model of allergic bronchopulmonary aspergillosis with elevated eosinophils and IgE. J. Immunol. 148, 3783–3788.

Kurup, V.P., Seymour, B.W.P., Choi, H., Coffman, R.L., 1994. Particulate *Aspergillus fumigatus* antigens elicit a T_{H2} response in BALB/c mice. J. Allergy Clin. Immunol. 93, 1013–1020.

Kurup, V.P., Choi, H., Murali, P.S., Resnick, A., Fink, J.N., Coffman, R.L., 1997. Role of particulate antigens of *Aspergillus* in murine eosinophilia. Int. Arch. Allergy Immunol. 112, 270–278.

Kusne, S., Torre-Cisneros, J., Manez, R., Irish, W., Martin, M., Fung, J., Simmons, R.L., Starzl, T.E., 1992. Factors associated with invasive lung aspergillosis and the significance of positive *Aspergillus* culture after liver transplantation. J. Infect. Dis. 166, 1379–1383.

McWhinney, P.H.M., Kibbler, C.C., Hamon, M.D., Smith, O.P., Gandhi, L., Berger, L.A., Walesby, R.K., Hoffbrand, A.V., Prentice, H.G., 1993. Progress in the diagnosis and management of aspergillosis in bone marrow transplantation: 13 years experience. Clin. Infect. Dis. 17, 397–404.

Morrison, V.A., Haake, R.J., Weisdorf, D.J., 1994. Non-candida fungal infections after bone marrow transplantation: risk factors and outcome. Am. J. Med. 96, 497–503.

Nagai, H., Guo, J., Choi, H., Kurup, V., 1995. Interferon-γ and tumor necrosis factor-α protect mice from invasive aspergillosis. J. Infect. Dis. 172, 1554–1560.

Puccetti, P., Mencacci, A., Cenci, E., Spaccapelo, R., Mosci, P., Enssle, K.H., Romani, L., Bistoni, F., 1994. Cure of murine candidiasis by recombinant soluble interleukin-4 receptor. J. Infect. Dis. 169, 1325–1331.

Rex, J.H., Benett, J.E., Gallin, J.I., Malech, H.L., DeCarlo, E.S., Melnick, D.A., 1991. *In vivo* interferon-γ therapy augments the *in vitro* ability of chronic granulomatous disease PMNs to damage *Aspergillus* hyphae. J. Infect. Dis. 163, 849–852.

Rinaldi, M.G., 1983. Invasive aspergillosis. Rev. Infect. Dis. 5, 1061–1077.

Roilides, E., Uhlig, K., Venzon, D., Pizzo, P.A., Walsh, T.J., 1993. Enhancement of oxidative response and damage caused by human PMNs to *Aspergillus fumigatus* hypae by granulocyte colony-stimulating factor and gamma interferon. Infect. Immun. 61 (4), 1185–1193.

Romani, L., Mocci, S., Bietta, C., Lanfaloni, L., Puccetti, P., Bistoni, F., 1991. T_H1 and T_H2 cytokine secretion patterns in murine candidiasis: association of T_H1 responses with acquired resistance. Infect. Immun. 59, 4647–4654.

Romani, L., Mencacci, A., Grohmann, U., Mocci, S., Mosci, P., Pucetti, P., Bistoni, F., 1992. Neutralizing antibody to interleukin-4 induces systemic protection and T helper type 1-associated immunity in murine candidiasis. J. Exp. Med. 176, 19–25.

Rosenberg, M., Patterson, R., Mintzer, R., Cooper, B.J., Roberts, M., Harris, K.E., 1997. Clinical and immunologic criteria for the diagnosis of allergic bronchopulmonary aspergillosis. Ann. Intern. Med. 86, 405–414.

Saxena, S., Ghosh, P.C., 1998. Pharmacokinetics of amphotericin B delivered through cholesterol hemisuccinate vesicles in normal and *A. fumigatus* infected mice. J. Antimicrob. Chemother., in press.

Saxena, S., Khan, J.A., Ghosh, P.C., 1998. Toxicity and therapeutic efficacy of amphotericin B delivered through cholesterol hemisuccinate vesicles in treatment of experimental murine aspergillosis. J. Antimicrob. Chemother. 42, 635–642.

Walsh, T.J., Pizzo, P.A., 1994. Aspergillosis. In: Hoeprich, P.D., Jordan, C., Ronald, A.R. (Eds.), Infectious Diseases, 5th edn. Lippincott, Philadelphia, PA, pp. 541–547.

Wang, L.H., Fielding, R.M., Smith, P.C., Guo, L.S.S., 1995. Comparative tissue distribution and elimination of amphotericin B colloidal dispersion (Amphocil) and fungizone after repeated dosing in rats. Pharm. Res. 12, 275–283.