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BRIEF REPORT

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The role of experimental aluminum intoxication in allogeneic immunoresponse

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Abstract To evaluate the immunological properties of aluminum (Al) in experimental Al intoxication in rats, we performed heart transplantation and in vitro experiments. Lewis (Lew) rats were intoxicated with intraperitoneal injections of $AlCl_3$. Heart transplants were performed using Brown-Norway (BN) rats as donors. Isotransplants and normal Lew were used as controls. No differences in survival were observed. Unidirectional mixed lymphocyte cultures (MLC) and Concanavalin A (Con A)-stimulated cultures were prepared using spleen cells from normal and Al-intoxicated Lew rats. No differences were found in unidirectional MLC. Intoxicated cells showed a less intense response to Con A than did normal cells. In conclusion, we could not detect an immunosuppressive role of Al intoxication in experimental cardiac transplantation or in MLC. However, the depressed Con A blastogenic response of Al-intoxicated cells may reflect an immunological role yet to be defined.

Key words Aluminum, immunoresponse · Immunoresponse, aluminum · Heart transplantation, rat, aluminum

Introduction

Aluminum (Al) intoxication can occur in chronic renal failure [3] by two mechanisms: ingestion of aluminum hydroxide as phosphate chelator [11] and Al contamination of water used in dialysis [1]. Other Al sources, such as parenteral solutions and blood transfusions, may also contribute to contamination [2, 4].

Davidson and Giles [6] noticed in 1979 a high rate of success in renal transplantation in patients with dialysis dementia, which is a manifestation of Al intoxication. Later, Nordal et al. [16] suggested a possible immunosuppressive role of Al and reported and inverse correlation between the number of rejection crises and Al intoxication in renal transplantation. This occurred independently of HLA matching, age, number of previous blood transfusions, or clinical status. Davenport et al. [5] also supported this hypothesis and reported an association between a low number of rejection crises and high Al excretion after renal transplantation.

The aim of the present study was to evaluate the immunological properties of Al in experimental Al intoxication in rats.

Materials and methods

Isogenic Lewis (Lew) ($RT1^{l,l}$), Brown-Norway (BN) ($RT1^{n,n}$), and Lewis x Brown-Norway hybrid (Lew x BN) F1 ($RT1^{l,n}$) (BNF1) male rats weighing 280–300 g were used. All animals received standard food and were maintained under the same environmental conditions. Lew rats were divided into two groups: nonintoxicated controls and Al-intoxicated animals. BNF1 rats served as donors of heart transplants and BN rats served as donors of stimulating cells in mixed lymphocyte cultures (MLC).

A 0.25 M $AlCl_3$ solution was used to obtain Al intoxication. The solution was injected into the peritoneal cavity through the median abdominal midline with a tuberculin syringe, once a day, 5 days a week. The initial dose (3 mg elemental Al/Kg) was increased gradually to 7 mg/kg until a total dose of 30 mg Al was injected. Control animals received acid saline.

Hearts were transplanted into the peritoneal cavity as described previously [17]. Graft survival was controlled by daily ab-

dominal palpation. Rejection was diagnosed when the heart beating stopped. Lew animals were divided into three groups. Group 1 consisted of five allotransplanted, Al-intoxicated rats, group 2 of seven allotransplanted, non-Al-intoxicated rats (allogenic controls), and group 3 of five isotransplanted, non-Al-intoxicated rats (isogenic controls).

Transplanted and native hearts, spleens, livers, and kidneys from intoxicated animals were collected, fixed in formalin, embedded in paraffin, and stained with hematoxylin-eosin for histological evaluation. These models were evaluated and approved by the Research Committee of the Department of Internal Medicine, Division of Nephrology, of the University of São Paulo School of Medicine.

The concentrations of Al in the nutrient medium used in culture experiments and in the serum and tissues of intoxicated and nonintoxicated Lew rats were measured by electrothermal atomic absorption spectroscopy [18]. The bones (left tibiae) were fixed in ethanol and embedded in methylmetacrylate and stained with aluminon [8] and solochromoazurine [10] for histochemical evaluation.

Unidirectional mixed lymphocyte cultures (UMLC) were prepared using lymphocytes harvested from the spleen. Mononuclear cells were isolated by density centrifugation with Ficoll-Hypaque (Sigma Chemical, USA). BN lymphocytes were irradiated with 4500 rads and were then cocultivated with nonintoxicated Lew or intoxicated Lew cells. The tests were performed in triplicate cultures in flat-bottomed wells of microtiter plates using 2.0×10^5 BN stimulator cells and 2.0×10^5 intoxicated or nonintoxicated Lew responder cells. Cells were cultured in RPMI-1640 medium (Flow Laboratories, USA), supplemented with 10% fetal calf serum (FCS; Cultilab, Brazil) and 1% 2-mercaptoethanol (Merck, Germany), and incubated in a humidified atmosphere of 5% CO_2 and 95% filtered air. The cultures were harvested on the 5th day after a 20-h pulse with ^{3}H-thymidine (Amersham International, UK) and processed with an automatic cell harvester.

Lymphocyte cultures with Concanavalin A (Con A; Sigma Chemical, USA) were prepared, also using spleen cells from intoxicated and nonintoxicated Lew rats (4×10^5 cells/well). The cells were dispersed into flat-bottomed well of microtiter plates and the mitogen was added to obtain a final concentration of 5 or 10 μg/ml Con A. The cell cultures were incubated at 37 °C in a humidified atmosphere of 5% CO_2 in air for 72 h. The cells were pulsed for 20 h with 1 μCi of ^{3}H-thymidine and harvested; thymidine incorporation was determined with a beta counter.

The Mann-Whitney U-test was used for statistical analysis.

Results

No difference was observed in allogenic graft survival between intoxicated and nonintoxicated recipients ($P = 0.41$). Results are summarized in Table 1.

No histological abnormalities were observed in native hearts, isotransplanted hearts, livers, spleens, or kidneys. Allotransplanted hearts (groups 1 and 2) showed hyperacute rejection with necrosis of cardiac tissue.

A positive staining reaction for Al was obtained in 80% and 89% of trabecular bone in aluminon and solochromoazurine-stained preparations from intoxicated rats, respectively. Non intoxicated animals showed negative staining reactions.

Al concentration was significantly lower in tissues from non-intoxicated Lew rats than in those from intoxicated animals ($P < 0.05$). Al concentration was 2.56 ± 0.25 μg Al/g tissue in the spleen of nonintoxicated Lew rats and 60.80 ± 11.37 μg Al/g in intoxicated rats. In the bones and liver of non-Al-intoxicated Lew rats, the Al concentrations were 1.24 ± 0.09 μg Al/g tissue and 0.39 ± 0.13 μg Al/g tissue, respectively, significantly lower values than observed in intoxicated animals (98.30 ± 12.38 μg Al/g tissue and 34.98 ± 1.69 μg Al/g tissue, respectively). Al concentration was 7.5 ± 0.6 μg/l in RPMI and 20.0 ± 2.3 μg/l in RPMI plus 10% FCS.

Table 1 Graft survival

Group	*n*	Survival (days)	Mean (days)
1	5	7,7,12,9,7	8.75 ± 2.01
2	7	6,7,7,7,7,8,9	7.28 ± 0.95
3	5	All > 90	> 90

$P < 0.03$ between group 3 and groups 1 and 2; no statistical difference between groups 1 and 2

Table 2 Blastogenic response to Concanavallin A

Lew rats	*n*	Con A 5 μg/ml (ΔCPM)	Con A 10 μg/ml (ΔCPM)
Nonintoxicated	6	320009 ± 18484	292013 ± 16025
Al-intoxicated	4	189187 ± 45565	177416 ± 60153
P		< 0.001	< 0.02

No statistical difference in blastogenic responses was observed between lymphocyte cultures from intoxicated LEW x BN irradiated rats ($n = 5$) and cultures from nonintoxicated LEW x BN irradiated rats ($n = 8$). The values obtained were 6860.66 ± 2714.97 ΔCPM and 7351.80 ± 4681.77 ΔCPM, respectively ($P = 0.89$).

Both lymphocytes from intoxicated Lew ($n = 4$) and nonintoxicated Lew ($n = 6$) showed a blastogenic response to Con A. However, cells from intoxicated animals showed a significantly less intense response than cells from nonintoxicated animals when 5 μg/ml or 10 μg/ml of Con A was added. In 5 μg/ml cultures, the results were 320009.0 ± 18484.53 ΔCPM in non-Al-intoxicated rats versus 189187.0 ± 45565.71 ΔCPM in Al-intoxicated rats ($P < 0.001$). In 10 μg/ml cultures, the results were 292013.5 ± 16025.61 ΔCPM for non-Al-intoxicated rats and 177416.25 ± 60153.54 ΔCPM for intoxicated rats ($P < 0.001$); Table 2). A significant difference ($P < 0.02$) was maintained when more experiments were done using only a single dose of Con A (5 μg/ml). Intoxicated Lew ($n = 8$) and nonintoxicated Lew ($n = 14$) cells showed 272404.71 ± 68361.75 ΔCPM and 184343.5 ± 47063.36 ΔCPM. This difference was not seen in control cultures to which no Con A was added.

Discussion

Al intoxication has been suggested to be an immunosuppressive factor that impairs lymphocyte function, thus permitting a better kidney survival [5, 6, 16]. In our experiments, allograft survival was not enhanced in Al-intoxicated rats, as demonstrated by positive Al staining in bones and high levels of Al in tissues and serum. It should be pointed out that, in our experimental model, Al was injected intraperitoneally. Part of $AlCl_3$ might be hydrolyzed to $Al(OH)_3$, which is a known immunological adjuvant that may stimulate the liberation of interleukin-1 by monocytes [9, 13]. This may act as an immunostimulant, counteracting the immunosuppressive properties of Al in intoxicated, transplant rats.

In addition, there were no significant differences in lymphoproliferative response in unidirectional mixed cultures. Nevertheless, in Con A experiments, there was a significant decrease in the blastogenic response to Con A of Al-intoxicated lymphocytes compared to controls.

There are some possible explanations for these data. One of them is that membrane or cell dysfunction can be observed only when the response is amplified by polyclonal lymphocyte stimulation. The second possibility is that Al may act as a Con A lymphocyte membrane blocker. We may speculate, for instance, that calcium is needed to bind Con A to lymphocyte receptors. It has been reported that Al can substitute for calcium in chemical reactions and in cellular membrane [7].

Some authors have demonstrated a reduced lymphoproliferative response to mitogen when Al-transferrin is added to human lymphocyte cultures [14]. Patients with chronic renal failure have high intracellular concentrations of Al [12]. However, lymphocytes from chronic renal failure patients with a high level of Al in serum have the same in vitro response to mitogen as normal controls [15]. These authors propose that Al may not induce lymphocyte malfunction in resting lymphocytes but rather may have a modulating effect on the blastogenic response to a mitogen, probably due to enhanced expression of transferrin receptor and to the internalization of the Al-transferrin complex present in high levels.

In summary, we could not detect an immunosuppressive role of Al intoxication in experimental cardiac transplantation or in in vitro mixed lymphocyte cultures. Al alone was unable to modify the immunoresponse. However, Al depressed blastogenic response to Con A, a fact possible reflecting an immunological role of the metal yet to be defined.

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