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Macrophage Effector Functions Controlled by Bruton’s Tyrosine Kinase Are More Crucial Than the Cytokine Balance of T Cell Responses for Microfilarial Clearance 1

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Macrophages from X-linked immunodeficient (xid) mice lacking functional Bruton’s tyrosine kinase (Btk) show poor NO induction and enhanced IL-12 induction, and contribute to delayed clearance of injected microfilaria (mf) in vivo. We now show that Btk is involved in other macrophage effector functions, such as bactericidal activity and secretion of proinflammatory cytokines (TNF-α, IL-1β), but not the T cell-directed cytokine IL-12. Induction of some transcriptional regulators of the NF-κB family, crucial for the expression of proinflammatory cytokines, is also poor in Btk-deficient macrophages. Thus, Btk appears to be involved in signaling for inducible effector functions, but not APC functions, in macrophages. Furthermore, adoptive transfer of T cells from mf-infected xid or wild-type mice did not alter the course of mf clearance in recipients, mf clearance was unaltered in IFN-γ-deficient mice, and improved mf clearance was seen only if greater inducibility of IL-12 was accompanied by greater NO secretion from macrophages, as seen in Ity’ C.D2 mice as compared with congenic Ity’ BALB/c mice. Thus, delayed mf clearance in xid mice was correlated not with the high IL-12/Th1 phenotype but with low NO induction levels. Also, xid macrophages showed poor toxicity to mf in vitro as compared with wild-type macrophages. Inhibition of NO production blocked this mf cytotoxicity, and an NF-κB inhibitor blocked both NO induction and mf cytotoxicity. Thus, Btk is involved in inducing many macrophage effector functions, and delayed mf clearance seen in Btk-deficient xid mice is due to poor NO induction in macrophages, resulting in compromised microfilarial toxicity. The Journal of Immunology, 2002, 168: 2914–2921.

The role and nature of immune-mediated protection in metazoan infections such as filariasis are still unclear (1–6). The role of the Th1/Th2 cytokine balance in the T cell response and the relative role of the innate and adaptive immune responses are major issues of debate (7–14), particularly because T cell cytokines (15–17) as well as certain innate immune mechanisms (14, 18–20) have been suggested to be trophic for filarial development as opposed to protection. Animal model-based studies of antifilarial immunity have been compromised by the fact that fully susceptible animal models such as gerbils, jirds, multimammate rats, or larger animal species (21) are not yet easily amenable to detailed immunological analyses.

Immunological studies performed in immunocompromised mice have been very useful in dissecting the nature of immunity in experimental filariasis, notwithstanding the fact that even such mice are not always fully susceptible to development of filarial parasites (21, 22). However, in immunocompetent mice, i.p. injected microfilaria (mf)4 can survive in peripheral blood for about 4 wk (23), although in T cell-deficient nude or SCID mice they survive for much longer times, establishing that active immunity is responsible for accelerated clearance, although the role of various components of the immune system in such clearance is still unclear.

We have reported previously that X-linked immunodeficient (xid) mice lacking functional Bruton’s tyrosine kinase (Btk) show prolonged persistence of circulating mf (16). Anti-mf T cell proliferative responses are enhanced, and the T cell cytokine pattern is skewed in the Th1 direction with relative dominance of IFN-γ in xid mice in comparison to wild-type mice (16). This bias is due to decreased induction of inducible NO synthase (iNOS) in the xid macrophages due to the absence of functional Btk, leading to de-repression of IL-12 induction, in turn causing the observed Th1 cell cytokine bias (24).

What is the role of the observed Th1 bias in anti-mf T cell responses found in xid mice in their inability to clear mf rapidly? There is some controversy about the precise role of the T cell cytokine balance in filarial infection. The T cell responses of asymptomatic humans with microfilaraemia show a prominence of Th2 cytokines such as IL-4, while T cells from symptomatic patients of elephantiasis show a relative dominance of the Th1 cytokine IFN-γ, as do some putatively immune “endemic normal” individuals (2, 12). However, Th2 responses appear to be protective in some experimental animal models (10, 25–27).

In contrast, our previous data also show that some macrophage effector functions may be compromised in Btk-deficient macrophages, because NO induction is severely inhibited (24). The mf

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4 Abbreviations used in this paper: mf, microfilaria; AG, aminoguanidine; Btk, Bruton’s tyrosine kinase; iNOS, inducible NO synthase; NRAMP-1, natural resistance associated macrophage protein-1; xid, X-linked immunodeficient.
are highly susceptible to NO and peroxynitrite-mediated damage in vitro (28), and mf clearance in mice can be delayed by administration of aminoguanidine (AG) (29), an inhibitor of iNOS (30, 31). However, we have also observed that NO negatively regulates IL-12 induction (24), and it therefore remains possible that the role played by macrophage NO in the mf clearance defect in *xid* mice is connected to Th1 cytokine biases rather than to macrophage effector functions.

On this background, we have investigated the effect of Btk deficiency on macrophage effector functions as well as the roles played by Th1 anti-mf T cell responses, particularly with reference to the role of IFN-γ, in mf clearance. We show in this study that the induction of macrophage effector functions is selectively inhibited by Btk deficiency, and, while mf clearance rates remain unaffected by Th1 cytokine patterns or the lack of IFN-γ, they are critically dependent on macrophage effector functions, particularly on NO induction.

### Materials and Methods

#### Animals

BALB/c, CBA/J, CBA/N, DBA/2 wild-type, and DBA/2 IFN-γ−/− mice (The Jackson Laboratory, Bar Harbor, ME) and C.D2 mice (gift of Dr. E. Skamene, University of Montreal, Montreal, Canada), bred and maintained in the Small Animal Facility of National Institute of Immunology (New Delhi, India), were used for experimental studies. Mice were used at 6–12 wk of age, and all experiments were done with the approval of the Institutional Animal Ethics Committee.

#### Parasites

*Setaria digitata* adult worms were collected from the peritoneal cavity of slaughtered cattle. Live mf of *S. digitata* were harvested in vitro from gravid female worms. Mice were challenged i.p. with mf (1 × 10^2–1 × 10^6 mf per mouse) and the clearance of mf from peripheral blood was monitored by counting mf twice a week in 10- to 20-μl Giemsa-stained thick blood smears.

#### Preparation of mf Ags

An aqueous extract of mf Ags was prepared by sonicating 1 × 10^9 mf in PBS (12 cycles of 4 min each) followed by centrifugation at 10,000 rpm for 10 min. The supernatant, referred to as mf sonicate, was kept as aliquots in liquid nitrogen until further use.

#### Macrophage stimulation assays

Bone marrow macrophages were grown by culture of bone marrow cells in M-CSF-containing L929-conditioned medium (30% final concentration) for 8–12 days as described earlier (32). Peritoneal exudate cells were harvested from mice as described earlier (24). Plastic-adherent macrophages from these cells were stimulated for 48 h with titrating doses of either bacterial LPS (*Salmonella typhosa*; Sigma-Aldrich, St. Louis, MO), or recombinant mouse IFN-γ (Genzyme, Boston, MA). Where indicated, macrophages were pretreated with the iNOS inhibitor AG (Sigma-Aldrich) for 60 min before stimulation. Culture supernatants were harvested from these cultures after 48 h for assaying induction of cytokines and NO.

#### Cytokine assays

The cytokines TNF-α, IL-1β, and IL-12 in the macrophage culture supernatants were quantified by two-site sandwich enzyme-linked immunoassay according the manufacturer’s protocols (BD PharMingen, San Diego, CA). Standard curves for the cytokines were obtained using recombinant standard proteins.

#### Estimation of nitrite accumulation

NO, produced by activated macrophages, reacts with oxygen to form nitrite and nitrate. As an indication of NO induction, the nitrite concentrations in the culture supernatants were measured using the Griess reaction (33) and quantified using a standard curve of sodium nitrite.

#### Western blot analyses

Western blot analysis was used to compare the induction of both cellular iNOS protein levels and nuclear levels of proteins of the NF-κB family in macrophages stimulated with LPS (10 μg/ml) for 36–48 h. Cell lysates or nuclear extracts were prepared as described (34, 35), centrifuged at 20,000 rpm (Beckman Coulter, Fullerton, CA), dialyzed, and stored at −70°C. Cell lysates or nuclear extracts (20 μg protein/lane) were separated in a 10% SDS-PAGE under reducing conditions. Following electrophoretic transfer to nitrocellulose membranes, incubations were done with affinity-purified goat Abs to the NF-κB family proteins c-rel, p65, or p50 (Santa Cruz Biotechnology, Santa Cruz, CA) followed by anti-goat Ig-HRP conjugate (Sigma-Aldrich). Blots were developed by chemiluminescence using manufacturer’s protocols (Amersham, Little Chalfont, U.K.). Images were scanned and analyzed using National Institutes of Health Image shareware (National Institutes of Health, Bethesda, MD).

#### T cell adaptive transfer

Mice, *xid* or wild-type, were infected i.p. with 2 × 10^5 *S. digitata* mf and challenged i.p. after 15 days. Splenic T cells were purified over nylon wool columns (16) and transferred either into naive *xid* or wild-type recipients. Mice were challenged i.p. with mf and the clearance of mf from peripheral blood was monitored.

#### Bactericidal assay

Comparison of bactericidal activities between *xid* and wild-type mice was performed as described (36). In brief, peritoneal macrophages from either strain of mice were incubated in triplicate cultures at a ratio of 1:10 with live *Escherichia coli* oposinized in 10% anti-*E. coli* serum. After 10 min, cells were washed and incubated further for varying periods of time, with 10 μg/ml gentamicin included for killing extracellular cell-adherent bacteria. At various time points during this incubation, cell aliquots were lysed with sulfuric deoxycholate and 10-μl aliquots were transferred into 100 μl of 10% Luria-Bertani Alamar blue (BioSource International, Camarillo, CA) in 96-well plates. The Luria-Bertani Alamar blue cultures were incubated at 37°C and then the absorbance was measured at 570 nm. Numbers of live bacteria were calculated by running standard curves of known bacterial concentrations. Bactericidal activity was expressed as the percentage of bacteria alive at each point of incubation time with reference to the number of bacteria found at 0 h of incubation time.

#### Microfilarial toxicity assay

Actively motile *S. digitata* mf (∼1000/well) were incubated with 3–5 × 10^5 peritoneal macrophages from either *xid* or wild-type mice (three to five mice per group) for 48 h in 96-well tissue culture plates. Where appropriate, macrophages were pretreated with the various modulators shown for 1 h before addition of mf. Microfilarial toxicity was monitored by recording the percentage of motile mf by microscopic observation. At least 200 mf per well were counted. Irreversibility of microfilarial toxicity was confirmed by subsequent culturing of the mf in macrophage-free medium for a further 24 h.

#### Statistical analysis

Data were analyzed using Student’s *t* test wherever applicable.

#### Results and Discussion

Our previous studies have indicated that Ag-specific T cells respond equally well to Ag presentation by either *xid* CBA/N or MHC-matched wild-type CBA/J macrophages (16). LPS-mediated induction of IL-12 is better in *xid* than in wild-type macrophages as an indirect consequence of poor iNOS induction in *xid* macrophages (24). When macrophages from *xid* or wild-type mice were stimulated with bacterial LPS and levels of MHC and major accessory or costimulatory molecules were analyzed by flow cytometry, it was evident that the induction of MHC class I, MHC class II, CD24, CD40, CD80, and CD86 on macrophages was comparable to wild-type macrophages (data not shown). Thus, Btk appeared to have little participation in the induction of T cell priming functions in macrophages. In contrast, one major defect we have already reported in Btk-deficient macrophages is the poor induction of iNOS and NO (24), and it was thus of interest to ask whether other effector functions of macrophages were also affected by the absence of functional Btk in *xid* mice.
Btk-deficient macrophages show poor microbicidal functions

The microbicidal functions of Btk-deficient macrophages were therefore directly examined next. Macrophages from xid or wild-type mice were incubated with opsonized E. coli for 10 min and incubated for various times before being lysed. The numbers of surviving bacteria in the lysates were estimated by growth in Luria-Bertani Alamar blue (36). The bactericidal ability of xid macrophages was significantly poorer than that of wild-type cells (Fig. 1). Thus, in 2 h of incubation, the wild-type cells reduced bacterial viability by >98%, while more than one-third of the input bacteria were still alive in the xid macrophage cultures \((p < 0.01)\). By the end of 3 h practically all input bacteria were killed by the wild-type macrophages, but \(\sim 15\%\) of the input bacteria were still alive in cultures with xid macrophages \((p < 0.01)\).

It is noteworthy that the poor bactericidal activity in xid macrophages is also accompanied by a deficit in bacterial phagocytosis. Thus, at the end of 10 min of incubation with opsonized bacteria, bacterial numbers associated with xid macrophages were significantly lower (by \(31 \pm 2\%\)) than those with wild-type macrophages. This defect in phagocytosis was also observed in preliminary flow cytometric experiments (data not shown). Defective phagocytosis in Btk-deficient macrophages is interesting in light of the reported connections between Btk, Wiskott-Aldrich syndrome protein, Cdc42, and actin polymerization (37–39).

The induction of reactive intermediates such as NO or reactive oxygen intermediate species is critically important for macrophage-mediated microbicidal activity (40–42). We have already demonstrated the defective induction of NO in xid macrophages, and preliminary results indicate that reactive oxygen species induction is also poor in them (data not shown).

Poor induction of proinflammatory cytokines in xid macrophages

Upon stimulation, macrophages secrete TNF-\(\alpha\) and IL-1\(\beta\) (43, 44), two major proinflammatory cytokines that have profound consequences on the efficacy of the innate effector immune responses during clearance of infections (45–47). The expression of these cytokines depends critically on the activation of members of the NF-kB family of regulatory proteins (48, 49). The efficiency of induction of these cytokines in macrophages from xid or wild-type mice was examined by stimulating them in vitro with bacterial LPS and estimating these cytokines in the culture supernatants 48 h later. The levels of induction of both cytokines (TNF-\(\alpha\) and IL-1\(\beta\)) were higher in wild-type than in xid macrophages, while higher levels of IL-12 were induced from xid macrophages as we have reported earlier (Fig. 2). In multiple independent experiments, the concentration of LPS needed to generate equivalent induction went up by 6-fold for TNF-\(\alpha\) and 10-fold for IL-1\(\beta\) \((p < 0.001)\) for xid macrophages over wild-type cells. Because these proinflammatory cytokines are also known to be directly cytotoxic for a number of parasitic pathogens (45–47), it was possible that they could be contributing to the delayed mf clearance observed in xid mice (16, 50).

We have previously shown that the increased induction of IL-12 from xid macrophages is a consequence of the poor induction of iNOS and NO in these cells (24). It was therefore of interest to ask whether the decreased induction of TNF-\(\alpha\) and IL-1\(\beta\) was also similarly indirect and dependent on the induction of NO. However, the inclusion of the iNOS inhibitor AG (30, 31) in the LPS-activated macrophage cultures did not change the levels of TNF-\(\alpha\) or IL-1\(\beta\) induction in either wild-type or xid macrophages (Fig. 3, A and B), suggesting that the effect of Btk on TNF-\(\alpha\) and IL-1\(\beta\) induction was NO independent, unlike the effect on IL-12 induction, which was a consequence of poor NO induction (see Ref. 24 and Fig. 3C).

FIGURE 1. Poor microbicidal activity in xid macrophages compared with wild-type ones. Pooled peritoneal macrophages from either wild-type (CBA/J) or xid (CBA/N) mice were incubated with opsonized E. coli for 10 min. Extracellular bacteria were killed using gentamicin and the surviving bacteria in the macrophages of both the strains of mice at various time points were estimated from macrophage lysates. The data are shown as the percentage of bacteria at various time points of incubation with reference to the numbers found at 0 h of incubation in triplicate cultures (mean ± SE). Results are representative of four independent experiments.

FIGURE 2. Induction of TNF-\(\alpha\) and IL-1\(\beta\) is poorer from xid macrophages than from wild-type cells. Peritoneal macrophages from wild-type (CBA/J) or xid (CBA/N) mice were cultured with titrating doses of bacterial LPS for 48 h, and levels of TNF-\(\alpha\) (A), IL-1\(\beta\) (B), and IL-12 (C) induced were measured in the culture supernatants. The data are representative of three separate experiments.
The macrophages so far tested were peritoneal exudate cells elicited by thioglycolate broth. It was possible that the elicitation itself led to differences in the activation status of the macrophages obtained from xid vs wild-type mice. Therefore, we used bone marrow macrophages from either strain of mice and tested their responses to bacterial LPS in terms of the induction of NO, IL-12, and TNF-α. The Btk-deficient macrophage phenotype, characterized by low levels of NO and TNF-α and high levels of IL-12 induction, was observed in bone marrow macrophages as well (Fig. 4).

Because the NF-κB family of transcriptional regulators has been shown to be important for microbicidal activity as well as the induction of expression of both TNF-α and IL-1β (48, 49, 51), we examined whether xid macrophages also showed deficient induction of nuclear translocation of NF-κB. Three members of the NF-κB family, p50, p65, and c-rel, were examined by Western blot analyses of nuclear extracts from LPS-activated macrophages of either wild-type or xid mice. Low levels of p65 could be observed in nuclear extracts even in the absence of LPS stimulation, possibly because these were thioglycolate-elicited peritoneal macrophages. However, induction of all three members of the rel family could be clearly observed (~3- to 5-fold in multiple experiments) in the case of wild-type macrophages, while xid macrophages showed no induction of either c-rel or p65, although some degree of p50 induction could be observed (Fig. 5).

FIGURE 3. Regulation of induction of TNF-α and IL-1β by Btk in macrophages is independent of NO. Peritoneal macrophages from wild-type (CBA/J) or xid (CBA/N) mice were cultured with titrating doses of bacterial LPS for 48 h in the presence or absence of AG, and levels of TNF-α (A), IL-1β (B), and IL-12 (C) induced were measured in the culture supernatants. The data are representative of three independent experiments.

FIGURE 4. Induction of NO, IL-12, and TNF-α is poorer from xid bone marrow macrophages than from wild-type cells. Bone marrow macrophages from wild-type (CBAJ) or xid (CBA/N) mice were cultured with titrating doses of bacterial LPS for 48 h, and levels of NO (A), IL-12 (B), and TNF-α (C) induced were measured in the culture supernatants. The data are representative of two separate experiments.

FIGURE 5. Poor induction of rel family proteins in xid macrophages. Peritoneal macrophages from xid or wild-type (WT) mice were cultured in the presence or absence of 10 µg/ml bacterial LPS for 48 h, and nuclear extracts were examined for levels of p65, p50, and c-rel proteins by Western blot analysis. The data shown are representative of three separate experiments.
The presence or absence of Th1 cell responses has little effect on microfilarial clearance.

We have shown previously that macrophages from xid mice secrete much more IL-12 than those from wild-type mice do (24). We therefore asked whether the poor rate of mf clearance in xid mice was directly correlated with the presence of high levels of IL-12 per se. We have observed (Fig. 6A) that LPS stimulation induces higher levels (~10-fold shift of dose response curves in multiple experiments) of IL-12 from macrophages of C.D2 mice than from BALB/c macrophages. The two strains are congenic for the Ity/Bcg/lsh locus, which is controlled by the expression of the endolysosomal transporter natural resistance associated macrophage protein-1 (NRAMP-1) (52, 53). These enhanced levels of IL-12 induction in C.D2 macrophages were not associated with any reduction of nitrite accumulation, unlike in xid cells. In fact, nitrite accumulation was higher in LPS-stimulated Ity C.D2 macrophage cultures than in Ity BALB/c macrophage cultures (Fig. 6A), although the level of iNOS protein induction was equivalent between the two groups (Fig. 6B). This is possibly due to the role of the NRAMP-1 molecule in NO transport (54). Thus, while xid and C.D2 macrophages showed higher levels of IL-12 induction than their respective wild-type counterparts, xid cells also showed compromised iNOS induction while C.D2 cells did not. However, when the rate of mf clearance was compared between BALB/c and C.D2 mice, no significant differences could be seen at low input numbers of mf (2 × 10^5 mf/mouse), while C.D2 mice showed significantly faster clearance at higher input numbers (1 × 10^6 mf/mouse; Fig. 6C). Thus, the rate of mf clearance does not correlate with the levels of IL-12 induced but does correlate with NO levels induced. It was therefore possible that compromised effector functions in xid macrophages could be the major cause for poor mf clearance.

We have reported that anti-mf T cell responses in xid mice are skewed toward the production of Th1 cytokines such as IFN-γ (16). Because xid mice clear injected mf far more slowly than wild-type mice (16), it was possible that Th1 cell responses led to delayed clearance of introduced mf in xid mice. To examine whether Th1/Th2-dominated anti-mf T cell responses could themselves have any major effect on the rate of mf clearance, we purified T cells from mf-infected wild-type or xid mice at day 15 postinjection and transferred them into naive wild-type or xid mice. These recipients were then given mf and the kinetics of mf clearances were estimated over time. Wild-type mice showed unaltered rates of mf clearance whether they had been given T cells from mf-infected xid or wild-type donors, and xid recipients showed delayed clearance despite receiving T cells from wild-type mf-infected donors (Fig. 7A).

These data suggested that the T cell cytokine profile was not of crucial importance in causing the difference between mf clearance rates of wild-type vs xid mice. The issue of whether Th1 cytokines,
notably IFN-γ, were in themselves critically important in controlling the rate of mf clearance was further examined directly by using IFN-γ−/− mice. It has been shown earlier that IFN-γ−/− mice do not show any significant alteration in the rate of mf clearance (3). However, these data have been obtained using IFN-γ−/− mice from C57BL/6 background (3), and it is known that the C57BL/6 genotype is particularly resistant to filarial infections. Therefore, we used a sensitive genotype in which mf clearance is less rapid and examined the effect of the lack of IFN-γ in such a strain. We have observed that DBA/2 mice show a particularly slow rate of mf clearance (data not shown). Therefore, the mf clearance rate was compared between wild-type and IFN-γ−/− DBA/2 mice. Clearance of microfilaraemia was similar in both groups, suggesting that presence or absence of IFN-γ made little difference (Fig. 7B). These data further reinforce the relative lack of significance of particular T cell cytokines in controlling mf clearance.

Btk-deficient macrophages show poor microfilarial toxicity correlating with poor induction of NO

Because bactericidal activity was poorer in xid mice compared with wild-type mice, we examined whether cytotoxicity was also compromised for large extracellular parasites such as mf in Btk-deficient macrophages. When actively motile mf of S. digitata were incubated in vitro with wild-type macrophages for 48 h, over two-thirds of them were rendered nonmotile (Fig. 8A). However, if xid macrophages were used instead, over three-fourths of the mf retained excellent motility at the end of 48 h of coincubation (Fig. 8A), demonstrating that Btk-deficient macrophages show poor microfilarial toxicity.

It has been shown that injected L3-stage infective larvae of Brugia malayi develop and mature in mice administered with iNOS inhibitor AG (29). Also, there are reports that NO can mediate microfilarial cytotoxicity in vitro (28). The levels of NO induced in mf cocultures were significantly different between xid and wild-type cells (Fig. 8B). We examined whether this was a causal correlation by including the iNOS inhibitor AG in titrated concentrations in these cultures. Increasing AG concentrations led to decreasing macrophage toxicity against mf (Fig. 8A), in parallel with decreasing induction of NO levels (Fig. 8B). At 100 μM AG, no detectable NO was induced and mf motility was not significantly affected (Fig. 8). These data suggest that the major defect in xid macrophages for mediating mf clearance is the poor induction of NO.

Because the absence of Btk leads to poor induction of the NF-κB family of transcription factors (Fig. 5), we asked whether NF-κB induction was essential for the cytotoxicity of macrophages toward mf. When the NF-κB inhibitor caffeic acid phenethyl ester (55) was added to the macrophage-mf cocultures, there was a dramatic blockade of mf cytotoxicity, accompanied by a reduction of NO induction (Fig. 9), consistent with a crucial role for NF-κB in the expression of iNOS and the resultant mf toxicity exhibited by macrophages. These data suggest that Btk-mediated induction of NF-κB is crucial in mediating microfilarial functions of macrophages.

Together, our findings show that, in Btk-deficient mice, compromised iNOS/NO induction in macrophages appears to be critically important in causing delayed clearance of microfilaraemia, rather than the precise cytokine pattern of the T cell responses seen.

Our data also identify an interesting division of macrophage functions between those important for T cell activation such as Ag presentation, costimulation, or T cell-directed cytokines like IL-12 on the one hand and “innate effector” functions such as induction of reactive metabolites, proinflammatory cytokines, and microbicidal activity on the other. Btk appears to be largely irrelevant for the first category of functions, while it plays a major role for the

FIGURE 7. Clearance of mf is not affected by the presence of Th1 cells. A, Time course of microfilaraemia (mean ± SE) in groups (n = 5) of mf-inoculated wild-type (CBA/J) mice or xid (CBA/N) mice receiving T cells from mf-injected wild-type or xid mice as shown. B, Time course of microfilaraemia (mean ± SE) in groups (n = 10) of mf-inoculated wild-type (DBA/2) mice or DBA/2 IFN-γ−/− mice is shown. Data are representative of two to three independent experiments.

FIGURE 8. NO-dependent macrophage toxicity toward mf in vitro is poor in xid macrophages. Peritoneal macrophages from wild-type (CBA/J) or xid (CBA/N) mice were cocultured with active and viable S. digitata mf (1000 mf/well) in absence or presence of various concentrations of AG for 48 h, and the motility and viability of mf (A) as well as the nitrite accumulation in the culture supernatants (B) were estimated and are shown as mean ± SE in triplicate cultures. Data in A are expressed as the percentage of mf remaining viable in comparison to mf incubated without any cells. Results shown are representative of three independent experiments.
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