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In vitro atrazine exposure affects the phenotypic and functional maturation of dendritic cells

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Abstract

Recent data suggest that some of the immunotoxic effects of the herbicide atrazine, a very widely used pesticide, may be due to perturbations in dendritic cell (DC) function. As consequences of atrazine exposure on the phenotypic and functional maturation of DC have not been studied, our objective was, using the murine DC line, JAWSII, to determine whether atrazine will interfere with DC maturation. First, we characterized the maturation of JAWSII cells *in vitro* by inducing them to mature in the presence of growth factors and selected maturational stimuli *in vitro*. Next, we exposed the DC cell line to a concentration range of atrazine and examined its effects on phenotypic and functional maturation of DC. Atrazine exposure interfered with the phenotypic and functional maturation of DC at non-cytotoxic concentrations. Among the phenotypic changes caused by atrazine exposure was a dose-dependent removal of surface MHC-I with a significant decrease being observed at 1 μM concentration. In addition, atrazine exposure decreased the expression of the costimulatory molecule CD86 and it downregulated the expression of the CD11b and CD11c accessory molecules and the myeloid developmental marker CD14. When, for comparative purposes, we exposed primary thymic DC to atrazine, MHC-I and CD11c expression was also decreased. Phenotypic changes in JAWSII DC maturation were associated with functional inhibition of maturation as, albeit at higher concentrations, receptor-mediated antigen uptake was increased by atrazine. Thus, our data suggest that atrazine directly targets DC maturation and that toxicants such as atrazine that efficiently remove MHC-I molecules from the DC surface are likely to contribute to immune evasion.

Keywords: Atrazine; Pesticide immunotoxicity; Dendritic cells; Maturation; MHC-I

Introduction

Owing to its ability to control broadleaf weeds, atrazine [(2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine)], a broad-spectrum chloro-*s*-triazine herbicide, is one of the most widely used pesticides in the U.S. (Gianessi and Marcelli, 2000). Atrazine is the most frequently detected pesticide in ground and surface waters where it tends to persist for months (Koskinen and Clay, 1997; Dorfler et al., 1997), particularly in waters with neutral or slightly basic pH (Cohen et al., 1984). Although levels of atrazine in the water are typically in the low ppb range, they can reach several hundred micrograms per liter (Koskinen and Clay, 1997; Dorfler et al., 1997). Besides the possibility of chronic, low-level exposure to atrazine in individuals consuming atrazine-containing well water, farmers, pesticide applicators, and other individuals in the vicinity of ongoing pesticide application, including children, may also be intermittently exposed to much higher levels of atrazine during application. Between 1993 and 1997, 14% of the farmers and commercial applicators in North Carolina and Iowa enrolled in the Agricultural Health Study (AHS) experienced unusually high pesticide exposure, with the percentage among the commercial applicators in Iowa being even higher (22%). Atrazine was one of only five pesticides that accounted for the majority of high pesticide exposure events (Storm et al., 2004).

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Because of the atrazine's widespread use and the increased potential of exposure to it either chronically (water), or intermittently (during application), scientific interest pertaining to its toxic potential has expanded. Series of studies, including ours, have investigated effects of exposure to this herbicide on the nervous, endocrine, and reproductive systems (i.e., Cooper et al., 2000; Narotsky et al., 2001; Stoker et al., 2000, 2002; Rodriguez et al., 2005; Filipov et al., 2007; Coban and Filipov, 2007).

Importantly, several studies have investigated effects of exposure to this pesticide on the immune system. Thus, in a study mandated by the National Toxicology Program (NTP), 14-day oral gavage exposure to atrazine of adult female $B6C3F_1$ mice decreased spleen cell numbers and spleen and thymus weights (more sensitive than the spleen). Atrazine exposure did not affect the humoral immune response to a T-cell-dependent antigen, the cytotoxic T lymphocyte response, NK cell activity, or the proliferative response to the mitogens Con A and LPS. However, exposure to this herbicide resulted in a dose-dependent decrease in host resistance in the B16F10 melanoma challenge test (NTP, 1994). Similar findings, i.e., reduced thymus and spleen weights, spleen cellularity, fixed macrophage function, as well as the host resistance to B16F10 melanoma, with the host resistance being compromised at levels of exposure (25 mg/kg) where no gross pathological effects of atrazine were observed, were reported in a different recent study (Karrow et al., 2005). Along the same lines, a single dose of atrazine (100 to 500 mg/kg) decreased thymic and splenic cellularity, affected lymphocyte subpopulations in the thymus and spleen, as well as the antibody responses to keyhole limpet hemocyanin (KLH) and NK cell activity (Pruett et al., 2003). Atrazine's immunotoxicity was also evaluated in developmental exposure paradigms and, interestingly, atrazine decreased the primary antibody and DTH responses only in the male offspring of Sprague–Dawley rats (Rooney et al., 2003), whereas developmental exposure of BALB/c mice (a Th2-skewed strain) to atrazine resulted in a significant increase in the number of antigen-specific IgM secreting B cells in the spleen of adult male offspring, a finding suggestive of possible Th1/Th2-specific effect of developmental atrazine exposure on dendritic cells (DC; Rowe et al., 2006).

Few *in vitro* studies have also assessed the immunotoxic potential of atrazine. From these studies, it appears that PHA-stimulated T cell proliferation is compromised by atrazine exposure, but only at relatively high concentrations (Pistl et al., 2003). On the other hand, much lower concentrations of atrazine (0.03–3 μM) substantially decreased the production of IFN-γ, IL-5, and TNF-α by human PBMC (Hooghe et al., 2000), a heterogeneous cell population that contains DC and their myeloid progenitors, monocytes.

During the last decade, DC, a family of bone-marrow (BM)-derived antigen-presenting cells (APC) have come to be appreciated as critical controllers of the immune response. The unique capacity of DC to sample sites of pathogen entry, respond to microbial and viral signals, and activate naive T and B cells suggests a critical role for these cells in initiating anti-microbial and antiviral immunity (Steinman, 1991; Banchereau and Steinman, 1998; Palucka and Banchereau, 1999). Extensive work in rodents and humans has demonstrated that the potent accessory properties of DC depend on a process of maturation (Larsen et al., 1990; Winzler et al., 1997). Immature DC efficiently process native antigens, but are relatively ineffective in activating naive T cells (Cella et al., 1997a). In contrast, mature DC lose their capacity to efficiently process antigens, but have increased ability to activate T cells upon the first encounter with the antigen (Winzler et al., 1997; Cella et al., 1997b). In particular, DC can be triggered by proinflammatory stimuli such as TNF-α, IL-1, Toll-like receptor (TLR) agonists — double-stranded RNA, Poly I:C (TLR3) and LPS (TLR4) to mature and to upregulate adhesion and costimulatory molecules (CD40, CD80, CD86; De Smedt et al., 1996; Roake et al., 1995; Cella et al., 1997a,b; Tsujimoto et al., 2006).

Although earlier data suggested that migration of immature DC from the periphery to the T cell areas of the secondary lymphoid organs only occurred in response to an antigen, more recent works indicate that migration of DC that contains apoptotic bodies occurs constitutively in the apparent absence of microbial stimulation and may contribute to the peripheral tolerance mechanisms (Huang et al., 2000; Steinman et al., 2003; Reddy et al., 2002). Moreover, in addition to immature "tolerogenic" DC, mature but resting DC can also induce tolerance *in vivo* (Albert et al., 2001) or deletion of antigen-specific $CD4^+$ T cells *in vitro* (Finkelman et al., 1996; Hawiger et al., 2001; Probst et al., 2003). These data strongly imply that DC, depending on both their maturation status as well as their effector properties, induce immunity or tolerance through a process requiring a concerted action of multiple subsets of DC (Pulendran, 2004, 2005). Recent evidence also suggests that multiple subpopulations of DC differ in their phenotype, microenvironmental localization, migration potential, PRR expression, responsiveness to microbes, and finally their effector capacity to promote Th1, Th2 or regulatory type of T cell development (Pulendran, 2004, 2005; Reis e Sousa, 2006). Therefore, it is important to define DC not only in terms of their maturation phenotypic properties but also in terms of their ontogeny and their effector properties (Reis e Sousa, 2006).

However, the difficulties in preparing DC in sufficient numbers in a reasonably pure form and the short life-span of DC in culture have greatly hindered the progress of knowledge of DC biology. Therefore, the establishment of DC lines has facilitated the generation of large numbers of DC in different stages of differentiation. In general, two types of DC lines have been generated: (i) immortalized DC lines which do not require continuous stimulation with growth factors for their propagation, and (ii) growth factor-dependent DC lines (Paglia et al., 1993; Lutz et al., 1999). An important limitation of most immortalized DC lines is that they retain an immature phenotype and cannot be stimulated to acquire a fully mature status. In contrast, growth factor-dependent DC lines can be more easily induced to mature *in vitro* and thus can more closely mimic the *in vivo* behavior of DC (Girolomoni et al., 1995).