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Immunosuppressive Effect of Isopropanol: Down-Regulation of Cytokine Production Results from the Alteration of Discrete Transcriptional Pathways in Activated Lymphocytes

Olivier Décy, Damien Carignan, Manuel Caruso, and Pedro O. de Campos-Lima

Isopropanol (IPA) is widely used in household applications and constitutes a leading cause of acute alcohol intoxication second only to ethanol. Although the effects of ethanol on the immune system have been extensively studied, far fewer data are available on IPA. Given the structural similarity between the two molecules, we hypothesized that IPA could also have immunomodulatory properties. We report here that acute IPA exposure is detrimental to human T lymphocyte and NK cell activity in vitro in concentrations as low as 0.08 – 0.16% (13–26 mM). IPA treatment did not affect receptor-mediated early signaling but had a reproducible and dose-dependent effect on the nuclear translocation of NFAT and AP-1. Furthermore, we show in a model of acute IPA intoxication that animals became immunosuppressed as judged by their reduced ability to release IL-2 and IFN-γ in the serum in response to staphylococcal enterotoxin B. This effect was also associated to the down-regulation of TNF-α production and was sufficiently strong to rescue susceptible animals from enterotoxin-induced toxic shock. Our results suggest that IPA is potentially immunosuppressive to the adaptive and innate immune system and have broad significance given the exposure of the general population to this ubiquitous chemical. The Journal of Immunology, 2008, 181: 2348 –2355.

Short-chain alcohols have a multitude of biological effects, including cardiac and CNS depression. In addition, a considerable body of evidence indicates that ethanol is capable of modulating the immune function mediated by T cells, monocytes, macrophages, and neutrophils (1–4). Ethanol also inhibits the leukocyte/endothelial cell interaction, thereby limiting the inflammatory response (4). Although the in vitro and in vivo effects of ethanol have been well characterized, many fewer data are available on other alcohols. Isopropanol (IPA) exposure is the second most common cause of acute alcohol intoxication in North America with ~20,000 cases reported each year to poison centers (5). IPA is readily available to most consumers as rubbing alcohol and as an ingredient of hand-sanitizing gels and other commonly used household solutions. In addition, IPA is widely used in hospitals as an antiseptic for surgical scrub and for patient care. Occupational exposure can also occur in numerous industrial applications. Previous studies addressed the impact of IPA exposure on the CNS, general hematological parameters, carcinogenesis, vascular permeability, urinary system, reproduction, and development (6–9). However, no detailed analysis of the potential impact of IPA on the immune system is available.

Given the structural similarity between IPA and ethanol, we hypothesized that IPA could also have immunomodulatory properties. We report here that IPA is detrimental to human T lymphocyte and NK cell activity in vitro in concentrations as low as 0.08 – 0.16% w/v (or 13–26 mM). These results were further substantiated in a mouse model of acute IPA intoxication in which animals were immunosuppressed as judged by their reduced capacity to produce inflammatory cytokines. This immunosuppression was sufficiently strong to protect susceptible animals from superantigen-induced lethal shock. Our results have broad significance taking into account the potential exposure of the general population to this ubiquitous chemical.

Materials and Methods

Cell isolation, culture, activation, and proliferation analysis

Mononuclear cells were prepared from peripheral blood from healthy volunteers by density gradient centrifugation using Ficoll-Hypaque (GE Healthcare). Written informed consent was obtained from all donors. More than 95% pure populations of human NK cells (CD56+) and T cells (CD8â†’ CD4â‡’) were obtained by using Ab-based EasySep separation kits with magnetic nanoparticles according to the manufacturer's instructions (StemCell Technologies). Cells were kept in complete medium, which was RPMI 1640 (Invitrogen Canada) supplemented with 10% heat-inactivated FBS (BioCell). IPA was purchased from BDH.

In most experiments, T cells were activated for 5 h at 37°C with anti-CD3/CD28 Ab-coated magnetic beads (Invitrogen). When indicated, alternative T cell activation protocols were used: protocol a, pretreatment for 20 min on ice with 1 μg/ml mouse anti-human CD3 mAb (CD3-2; Mabtech) followed by washing and incubation for 3 min at 37°C with 10 μg/ml goat anti-mouse IgG (Invitrogen); protocol b, treatment with 10 ng/ml PMA (Sigma-Aldrich) and 200 ng/ml ionomycin (Sigma-Aldrich) for 5 h. Human NK cells were activated for 24 h by treatment with 50 μg/ml polyinosinic-polycytidylic acid (poly(IC); Sigma-Aldrich) in presence of 10 U/ml IL-2 and 0.01 ng/ml IL-12 (Feldan Bio).

CFSE staining

Freshly purified T cells were labeled with 10 μM CFSE (Invitrogen) in PBS, 1% FBS for 10 min at 37°C and further incubated in RPMI, 10% FBS for 5 min on ice; then cells were washed and activated with anti-CD3/CD28 Ab-coated beads for 5 h with or without 0.6% (w/v) IPA. The activating beads were magnetically removed, and the cells were washed and
incubated for 72 h in 96-well plates (10^5 cells/ml) in complete medium without exogenous IL-2. FACS analysis of cell divisions and surface marker expression was performed on a XL flow cytometer (Beckman Coulter).

Western blot and luciferase assay

**Western blot.** Purified T cells were activated for 3 min at 37°C by anti-CD3/anti-IgG Abs as described above with or without 0.6% (w/v) IPA. The cells were lysed in SDS sample buffer (2% w/v SDS, 0.25 M 2-ME, 10% v/v glycerol, 0.05 M Tris-HCl (pH 7.8), 0.004% w/v borophenon blue); lysates were separated in 12% polyacrylamide gels and blotted onto nitrocellulose filters (Hybond C; GE Healthcare). The membranes were first probed with ZAP70-specific Abs: rabbit anti-human ZAP70 (99F2, 1:1,000; Cell Signaling Technology); and mouse anti-human ZAP70 (pY319)/Syk (pY352) (17a, 1:5000; BD Biosciences); then they were washed and incubated with 1/15,000 dilutions of the Abs IRDye 800CW goat anti-rabbit IgG and IRDye 680 goat anti-mouse IgG (Li-Cor Biosciences). Detection and quantification were performed with the Odyssey Infrared Imaging System (Li-Cor Biosystems).

**Luciferase assay.** Jurkat-luc cells were stimulated with PMA-ionomycin with or without IPA treatment as indicated in the text. Lysates for luciferase assays were prepared with the passive lysis buffer (E1941; Promega), mixed with reaction solution (25 mM glycglycylglycine (pH 7.8), 10 mM MgSO4, 5 mM ATP), and analyzed in a Berthold Lumat 9501 luminometer (Berthold; 3 h after the addition of α-fucosin (Fischer Scientific Co.) to a final concentration of 0.1 mM. Relative luciferase units were calculated in relation to the unstimulated negative control after normalization to total protein content measured by the Bradford assay (Bio-Rad).

**Generation of Jurkat-luc cells.** Stable Jurkat-luc cells were generated by lentiviral transduction. Vector generation and transduction conditions: The synthetic promoter used in our studies contains three copies of the human distal IL-2 NFKAT-binding site placed upstream of the ~77 to ~45 region of the human IL-2 promoter. The firefly luciferase gene driven by this synthetic promoter was cloned in the Nhel and Xhol sites of a modified version of pRRL-5pme (10) to generate pLV-iluc. This parental version of the human IL-2 promoter. The firefly luciferase gene driven by this synthetic promoter was cloned in the Nhel and Xhol sites of a modified version of pRRL-5pme (10) to generate pLV-iluc. This parental version of pRRL-5pme also carries the ZOEcin resistance gene driven by the promoter of the human phosphoglycerokinase gene. Lentiviral supernatants were generated in 293T cells by transient transfection (11). Three plasmids, pMDLg/RRE, pRSV-rev, and pMD.VSV-G, were cotransfected with pLV-iluc. The supernatants containing lentivirus were harvested 48 and 72 h after transfection, filtered through a 0.45-μm pore size filter, and frozen at ~80°C until use. Jurkat cells were transduced overnight in 24-well plates at 2.5 x 10^5 cells/ml with 0.5 ml of viral supernatants plus 0.5 ml of fresh medium and 8 μg/ml polybrene (Sigma-Aldrich). Stable Jurkat-luc cells were generated after two rounds of 4-day ZOEcin selection (Invitrogen).

Cytokine analysis

Measurements of human (h) or murine (m) IL-2 and IFN-γ in cell culture supernatants and murine IL-2 and IFN-γ in serum samples were performed by ELISA-based transcription factor activation assay

**ELISA-based transcription factor activation assay**

Nuclear proteins were extracted using the Active Motif Nuclear Extract kit (Active Motif) according to the manufacturer’s instructions; the total protein concentration of the lysates was determined by the Bradford assay (Bio-Rad). NFKAT activation was measured with the TransAM NFKATel kit; c-Fos and c-Jun activation was measured with the TransAM AP-1 kit; p50 and p65 activation was measured with the TransAm NF-κB kit. ELISA-based transcription factor assays were used according to the manufacturer’s instructions (Active Motif). Briefly, nuclear extracts were incubated with plate-bound transcription factor-specific oligonucleotides, and the plates were washed and further incubated with transcription factor-specific Abs. Addition of a HRP-conjugated secondary Ab and the 3’,3’,5’,5’-tetramethylbenzidine substrate produced a colorimetric reaction measurable in a spectrophotometer.

Cytotoxicity assays

The cytotoxic activity was analyzed in standard 4-h 51Cr release assays as reported elsewhere (11). The targets were labeled with Na11032CrO4 for 2 h at 37°C. Tests were performed in the presence or absence of 0.6% (w/v) IPA in triplicate. Freshly purified NK cells and peripheral T lymphocytes were used as effectors. Target cells were the NK-sensitive K562 cell line and a control autologous lymphoblastoid cell line in the NK cells assays. Targets for T cell assays were the OKT3 hybridoma and a control hybridoma specific to MAGE-A9 (kindly provided by Dr. Alain Bergeron, Laval University, Quebec City, Canada).

In vivo studies

Female BALB/c mice (7–13 wk old) were bought from The Jackson Laboratory. All tests respected the ethical guidelines set by the Institutional Animal Protection Committee (CPA-Centre Hospitalier Universitaire de Québec, Quebec, Canada). Animals received 5 μg of staphylococcal enterotoxin B (SEB; Toxin Technology) s.c. for cytokine induction and were sacrificed by CO2 asphyxiation 2 or 4 h after administration for IL-2-TNFα or IFN-γ serum analysis, respectively.

For the analysis of murine T cell subsets, mice received 20 μg of SEB i.v. with (or without) an i.p. injection of 2 g/kg IPA. The animals were sacrificed 160 min later for spleen isolation; CD4+ and CD8+ T cell populations were purified by using Ab-based cell separation kits with magnetic nanoparticles (StemCell Technologies) according to the manufacturer’s instructions. Purified cells (97–98% pure) were cultured in vitro without IPA for 18 h, and the supernatants were checked for the presence of IFN-γ by ELISA.

Toxic shock was induced with a s.c. injection of 10 μg of SEB after presensitization with 20 μg D-galactosamine (Sigma-Aldrich) as reported elsewhere (12). IPA was injected i.p. (2 g/kg). Mice were checked hourly for 72 h. Animals that survived the 72-h experiment were followed for 5 days. The blood alcohol concentration was determined by gas chromatography with a 3900 GC unit (Varian).

Statistical analysis

One-way ANOVA followed by Dunnett’s multiple-comparison posttest was performed with GraphPad Prism (GraphPad Software) on data presented in all figures, except when indicated otherwise. Student’s t test was used in Figs. 2B and 4, C and D. Survival curves were determined by the Kaplan-Meier method (Fig. 5E). p values <0.05 were considered significant.

Results

**IPA interferes with the production of IL-2 and the proliferative capacity of Ag receptor-activated peripheral T lymphocytes**

The IL-2 gene is transcribed following Ag-specific activation of the TCR. In this study, we have investigated first whether IPA exposure in vitro would have any impact on the ability of human peripheral lymphocytes to produce IL-2 once activated by Ag cross-linking of the TCR. These cells produced less IL-2 when treated with IPA as measured in the culture supernatants by ELISA (Fig. 1A). The reduction in cytokine production was observed at IPA doses as low as 0.16%. The observed alcohol effect was not the consequence of nonspecific cytotoxicity given that the cell viability of IPA-treated samples in the concentration range that produced 36–86% IL-2 inhibition was similar to that of untreated control cells (Fig. 1A). Given the importance of the IL-2 autocrine loop for the expansion of Ag-specific cells in vivo, we asked whether the reduced production of IL-2 translates into a lower proliferative capacity of the activated lymphocytes. Purified T cells were labeled with CFSE, activated, and analyzed 72 h after by flow cytometry. Although the 5-h TCR cross-linking led to 1–4 divisions in ~40% of the cells in absence of exogenous IL-2 (mean ± SEM 39.69 ± 2.06, n = 4), cell proliferation was much less pronounced in presence of IPA treatment (mean ± SEM 18.82 ± 4.53, n = 4). The means of IPA-treated and untreated cells differ with a p value of
IPA blocks IL-2 production via transcriptional inhibition

The lack of an obvious impact of the IPA treatment on early TCR signaling led us to examine the possibility of a negative effect on IL-2 transcription. For this purpose, we have generated a stable Jurkat subline carrying the firefly luciferase gene driven by a synthetic IL-2 minimal promoter shown previously to be responsive to PMA-ionomycin (Jurkat-luc) (14, 15). IPA was capable of inhibiting in a dose-dependent manner the luciferase activity triggered by PMA-ionomycin in these cells (Fig. 3A). IPA concentrations as low as 0.3% had a significant dampening effect on IL-2 transcription as indicated by a 24% reduction in luciferase activity. Similar results were obtained by anti-CD3 Ab cross-linking in Jurkat-luc cells (data not shown).

Nuclear translocation of transcription factors is affected by IPA in activated T cells

The promoter used in the experiments shown in Fig. 3A contains three copies of the binding site for NFAT placed upstream of the IL-2 core promoter and is highly responsive to the Ca\(^{2+}\)-NFAT signaling pathway (14, 15). However, the regulation of the IL-2 gene following TCR triggering is more complex and involves the participation of transcription factors activated by two additional major signal transduction pathways (16). To dissect further the relative impact of IPA on these molecules, we have measured the nuclear translocation of NFAT (NFATc1), NF-xB (p50/p65), and AP-1 (c-Jun/c-Fos) in TCR-stimulated purified human T cells exposed to different concentrations of IPA. Fig. 3B shows that lymphocyte activation by anti-CD3/CD28 Abs led to a 2-fold increase in the amount of NFAT in the nucleus. The same stimulation in the presence of 0.6% IPA led to only a 1-fold increase in nuclear NFAT (or 54% of the maximal NFAT nuclear content above the unstimulated cell baseline). This effect was dose dependent with the highest inhibition observed for the highest IPA concentrations. The calcineurin inhibitor cyclosporin A was used as a control in the same stimulatory conditions with little variation in nuclear NFAT content (17% less than the nuclear content baseline of unstimulated cells). Activation of AP-1 in the presence of IPA followed the same pattern observed for NFAT with 55% of the maximal c-Jun nuclear content above the unstimulated cell baseline achieved at the highest IPA concentration (Fig. 3E). Activation by anti-CD3/CD28 Abs in the presence of the JNK inhibitor SP600125 produced 42% of the maximal c-Jun nuclear content above the unstimulated cell baseline. The same T-cell stimulatory conditions produced 45.4% of the maximal c-Fos nuclear content in presence of the highest IPA concentration and 88.3% in presence of the MEK1 inhibitor PD98059 (Fig. 3F). In contrast to the results obtained with NFAT and AP-1, activation of NF-xB remained unaffected by IPA treatment at all tested concentrations (Fig. 3, C and D). The compound BAY 11-7082, an inhibitor of IxB\(\alpha\) phosphorylation, reduced the nuclear content of p50/p65 in the same experiments to levels lower than those of the unstimulated control.

Production of the inflammatory cytokine IFN-\(\gamma\) by human peripheral T lymphocytes and NK cells is inhibited by IPA

The NFAT and AP-1 families of transcription factors play a major role in the expression of several cytokines, including IFN-\(\gamma\) (17–22). The finding that the inhibition of IL-2 production in IPA-treated T cells is associated with reduced nuclear translocation of c-Jun, c-Fos, and NFAT led us to speculate that IFN-\(\gamma\) expression would also be affected in these cells. First, we have examined the IFN-\(\gamma\) release in peripheral T lymphocytes following activation with anti-CD3/CD28 Abs in presence of...
different IPA concentrations (Fig. 4A). Treatment with 0.16% IPA led to a 35% reduction in IFN-γ release. The higher IPA concentrations tested, 0.3%, 0.6% and 1.2% produced inhibitory effects of 55, 84, and 98%, respectively. Cyclosporin A in the same experimental conditions led to a virtually complete IFN-γ inhibition (data not shown).

The above results have encouraged us to extend our analysis to another immune cell capable of producing large amounts of IFN-γ. Purified human NK cells have been stimulated in vitro with poly(I:C) in the presence of IL-2 and IL-12 and exposed to two highest IPA concentrations as low as 0.08% were active. Treatment with 0.08, 0.16, and 0.3% IPA reduced the IFN-γ release by stimulated NK cells by 31, 40, and 87%, respectively. The two highest IPA concentrations produced an almost complete inhibitory effect with only background levels of IFN-γ being released. NK cells were >95% viable at all IPA concentrations tested (data not shown).
IPA reduces the cytotoxic activity of T lymphocytes and NK cells in vitro

The identification of the negative impact of IPA on the production of IFN-γ by T and NK cells has prompted us to examine if this effect was extended to other effector functions. Fig. 4C shows that the cytotoxic activity of purified human peripheral T lymphocytes against OKT3 hybridoma cells was inhibited by ~20% in presence of IPA. OKT3 cells display the activating anti-CD3 Ab and work as a T cell target (23). Similarly, the cytotoxicity of purified human NK cells against K562 target cells was inhibited by 30–40% in the presence of IPA (Fig. 4D).

IPA inhibits the production of IL-2 and IFN-γ in vivo

After having demonstrated the negative impact of IPA treatment on IL-2 and IFN-γ production by lymphocytes in vitro, we have examined the relevance of these findings in a mouse model of acute IPA intoxication. Mice were treated with IPA, 2 g/kg i.p., to generate a mean blood alcohol concentration of 200 mg/dl after 30 min (198 ± 3.704, n = 10). Induction of IL-2 and IFN-γ production in vivo was achieved by s.c. injection of the superantigen SEB. T lymphocytes with the relevant TCR Vβ chains undergo transient activation and cell proliferation and begin massive cytokine production (12). As anticipated, injected SEB induced IL-2 levels of >12 ng/ml after 2 h (Fig. 5A) and >1.6 ng/ml IFN-γ after 4 h (Fig. 5B). IPA dampened the cytokine production substantially: 49.2% or 6.3 ng/ml IL-2 detected at 2 h; and 86.6% or 0.2 ng/ml IFN-γ detected at 4 h. The differences in cytokine serum levels between animals treated and untreated with IPA were statistically significant as indicated in Fig. 5, A and B.

IPA treatment impacts both CD4⁺ and CD8⁺ T lymphocytes in vivo

Both CD4⁺ and CD8⁺ T lymphocytes may respond to SEB to produce cytokines. We have examined next how these T cell subsets were affected by IPA in our in vivo experimental model. BALB/c mice were injected with SEB i.v. to activate CD4⁺ and CD8⁺ T cells carrying the responsive TCRs; IPA was provided i.p. It has been shown that a minimal T cell-APC conjugate time of 2 h is required for commitment to cytokine production and cell proliferation (24). On this premise, we have followed the animals for 160 min, after which they were sacrificed, and their splenocytes were harvested and separated into CD4⁺ and CD8⁺ T cell populations. Purified T cell subsets were incubated for 18 h at 37°C and culture supernatants were analyzed by ELISA for IFN-γ production. Fig. 5C shows that IPA exposure in vivo reduced the IFN-γ release by CD4⁺ T cells in vitro by 74%. Similarly, the IFN-γ production in vitro by CD8⁺ T cells decreased ~70% when animals from which they derived were exposed to IPA for 160 min (Fig. 5D).
The immuno-suppressive effect of IPA confers protection to animals from SEB-induced toxic shock

The ability of IPA to prevent or substantially reduce the production of cytokines in response to SEB in vivo led us to wonder whether this immuno-suppressive effect could delay the development of toxic shock in susceptible animals. To investigate this possibility, we have sensitized BALB/c mice with 20 mg of α-galactosamine by i.p. injection. All sensitized animals succumbed to toxic shock within 14 h after receiving SEB s.c. (12/12; Fig. 5E). In contrast, a single injection of 2 g/kg IPA had a substantial effect because it delayed or completely aborted the development of SEB-induced toxic shock in all animals. Seven of twelve animals survived the 72-h experiment; they were followed for up to 5 days after SEB injection and were indistinguishable from sensitized control groups receiving PBS or IPA as regards activity, grooming, eating, and drinking behavior. No animal injected with IPA (12/12) or PBS (12/12) in the absence of SEB died.

In addition to IL-2 and IFN-γ, the inflammatory cytokine TNF-α is copiously produced and plays a central role in the pathophysiology of superantigen-induced lethal shock (12). TNF-α gene transcription was shown by other investigators to be regulated by NFAT (25). Our results revealed IPA as a negative regulator of transcription was shown by other investigators to be regulated by NFAT (25). Our results revealed IPA as a negative regulator of NFAT nuclear translocation in vitro and as an immuno-suppressive agent capable of protecting mice from toxic shock. Therefore, it was a logical assumption that IPA treatment would block TNF-α production in vivo. Indeed, Fig. 5F shows that mice treated with SEB produced significant amounts of TNF-α in the first 2 h after injection as opposed to animals that received IPA + SEB.

Discussion

Alcohols have the ability to partition into cell membranes and to denature proteins by promoting the formation of α-helices and/or by disrupting tertiary structures; these effects are largely nonspecific and are typically observed at high concentrations (>2% w/v or >500 mM; Ref. 26). At more physiologically relevant concentrations, alcohols have been shown to induce loss of function of specific proteins, such as ion channels, neurotransmitter receptors, enzymes, and adhesion molecules (27–29). Structural and biophysical data suggest that binding to the target proteins occurs at discrete sites that are constituted by hydrophobic pockets lined by nonpolar amino acids (26–29). As suggested for other short-chain alcohols (26, 30), IPA could displace water molecules from such pockets and establish contact with the proteins via hydrogen bonds that would be stabilized by van der Waals forces in the hydrophobic region. These interactions would ultimately produce a local distortion and alteration in protein function.

Another interpretation for the effects of IPA would result from the possible interference with the capacity of membrane microdomains to recruit and/or retain molecules involved in signaling, thus compromising the formation of the immunological synapse. Given the central role played by lipid rafts in amplifying receptor-mediated signals in immune cells (3), it is conceivable that IPA could affect surface molecules, such as the TCR, directly by inducing unfavorable conformational changes or, indirectly, by disrupting lipid-protein interactions. A similar model has recently been evoked to explain the ethanol inhibition of LPS-mediated TLR4 signaling in macrophages (3).

Upon engagement of the relevant ligand, the TCR triggers a phosphorylation cascade that is followed by a biphasic increase in intracellular Ca²⁺. The initial wave derives from the intracellular stores and is rapidly trailed by the extra-cellular influx regulated by Ca²⁺ release-activated Ca²⁺ channels (13). We initially examined if IPA would exert its immuno-suppressive effect by interference with the Ca²⁺ release-activated Ca²⁺ channel-regulated Ca²⁺ influx and the subsequent calcineurin-dependent activation of NFAT. There was some support for this possibility given the reported association of other alcohols with ion channels, often altering their function (26–27, 29). We failed to show any IPA-induced change in the pattern of intracellular calcium increase that follows TCR triggering or ionomycin treatment (data not shown). The fact that TCR-mediated early signaling as measured by ZAP70 phosphorylation and calcium release is preserved indicates that the effect of IPA is downstream of the cell membrane. We cannot discard the idea, however, that higher IPA concentrations could also affect lipid rafts in a way reminiscent of the model suggested for ethanol on the TLR4 receptor (3), but this remains to be experimentally tested.

The inhibition reported here was observed in vitro at IPA concentrations as low as 0.08% (13 mM) as measured by IFN-γ release in NK cells and 0.16% (26 mM) as measured by IL-2 and IFN-γ release in T cells. These concentrations are equal to or lower than those of ethanol used in previous studies that reported a statistically significant impact on immune cells (1–4). Many of the biological effects of ethanol on the immune system have been associated to a reduced nuclear translocation of NF-kB, a transcription factor capable of binding the promoter regions of multiple cytokines (3, 4). In contrast, we found that IPA does not affect NF-kB but has a reproducible and dose-dependent effect on the nuclear translocation of AP-1 and NFAT. This finding supports the view that IPA exerts its impact on immune cells through the interaction with selective pathways rather than a membrane-based nonspecific down-modulation of the immune cell activation. NFAT and AP-1 have been shown to modulate synthesis of the three cytokines examined in this paper (IL-2, IFN-γ, and TNF-α) (14–15, 17–22, 25). As expected, NFAT nuclear translocation and cytokine release were blocked by cyclosporin A in activated lymphocytes. Nevertheless, IPA differed from cyclosporin A in that it did not target the calcineurin phosphatase activity (data not shown). Thus, it must interact directly with NFAT or with downstream molecules involved in its nuclear translocation, such as importin β1, or molecules involved in its phosphorylation in the nucleus, such as glycogen synthase kinase 3. Similarly, IPA may also interact directly with c-Jun and/or c-Fos compromising the formation and/or function of the AP-1 dimer as the phosphorylation pattern of upstream molecules such as p38 remains unchanged (data not shown).

To address the immuno-suppressive effects of IPA in an in vivo setting, we have used a model of acute intoxication. Deliberate or accidental ingestion of IPA ranks second as a cause of alcohol poisoning according to the 2005 annual report of the American Association of Poison Control Centers (5). Acute intoxication usually occurs in alcoholic patients, children, and suicidal individuals (31). The blood IPA concentration can reach levels as high as 560 mg/dl (0.56% or 93 mM); many of the reported measurements have been made hours after ingestion and may underestimate the serum amounts present in the early phase of the intoxication (32–37). Nevertheless, a concentration above 400 mg/dl is usually considered life-threatening and demands a more aggressive intervention such as dialysis (38–39). In our model, we have injected mice with IPA 2 g/kg i.p. to generate a blood alcohol concentration of 200 mg/dl (0.2% or 33 mM) after 30 min; this level is under the reported average sublethal blood concentration in intoxicated humans (310 mg/dl; Ref. 40) and is well within the concentration range that we have shown to be biologically active in vitro (starting at 0.08–0.16% or 13–26 mM). Our results indicate that during this state of acute intoxication the animals are immuno-suppressed as judged by their reduced ability to release IL-2 or IFN-γ in the...
serum in response to SEB. The magnitude of this immunosuppression was further assessed by monitoring the survival of animals injected with SEB after presensitization with β-galactosamine. We were initially uncertain about the outcome of this particular experiment because we thought that it might be too stringent. In fact, all presensitized animals developed a fulminating toxic shock syndrome with a median survival of 9 h after SEB injection. Nevertheless, in contrast to the untreated animals, the syndrome did not occur or had its development delayed in all mice treated with IPA, and the majority survived. This is in line with the massive suppressive effect of IPA on the production of IL-2, IFN-γ, and TNF-α. It is believed that the production of copious amounts of IFN-γ, and especially of TNF-α, plays a major role in the development of the syndrome (12, 25).

An obvious consequence of our findings is the assumption that any individual acutely intoxicated by IPA may also be acutely immunosuppressed. This assumption could be easily tested in a clinical setting and may provide the basis for the establishment of precautionary measures in the emergency room to deal with this predicament. The issue would be particularly relevant in circumstances in which an underlying infection or trauma complicate the clinical picture.

The implications of our results could be also extrapolated to areas other than acute systemic intoxication. IPA is used in many industrial applications, and occupational exposure by inhalation or other routes may occur (8). One of the major weaknesses of the literature on IPA is the virtual absence of solid information about chronic toxicity in humans. Our experiments were not designed to address this issue. Nevertheless the results reported here for acute exposure indicate that immunological parameters may serve as a sensitive endpoint for IPA toxicity that could be included in future studies on the long-term effects of this chemical.

Intact adult skin is not an efficient route of IPA absorption; the skin permeation coefficient (kP) is estimated to be in the order of 4–15 × 10⁻⁴ cm/h (41–43). Yet, dermal absorption does occur (41, 44, 45), and a few cases of systemic IPA intoxication after topical exposure have been reported in the literature (46–48). IPA is present, often in a high concentration (60–95%), in hand sanitizer gels/solutions and many household products readily available over the counter. Taking into consideration that our results show a significant biological effect in vitro with IPA concentrations as low as 0.08–0.16% (13–26 mM), it is reasonable to question whether the application to the skin of a product that is 500–1000 times more concentrated would have similar consequences even in the context of poor dermal absorption. As regards intact normal adult skin, it is likely that the immunosuppressive effect, if any, would be transitory and only relevant to the immune cells present in the treated skin itself. Nevertheless, future studies to address this issue are warranted given the widespread and poorly regulated use of this chemical.

The in vitro data presented here suggest that acute IPA exposure reduces the ability of lymphocytes to produce proinflammatory cytokines, and thus may compromise the innate and adaptive immune system; in addition, acute intoxication led to acute immunosuppression in vivo, an effect that was sufficiently strong to rescue susceptible animals from enterotoxin-induced toxic shock. These results are directly relevant in the context of acute IPA intoxication and constitute a rational for the inclusion of immunological endpoints into the design of future studies to address chronic and topical IPA exposure.

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Disclosures

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