A symposium entitled Alterations in Cytokine Receptors by Xenobiotics was held at the 37th Annual Meeting of the Society of Toxicology (SOT) in Seattle, Washington. The symposium was sponsored by the Immunotoxicology Specialty Section of SOT and was designed to present information on the effect of several different classes of xenobiotics on various aspects of receptor function (i.e., post-receptor signal transduction of receptor expression), or the involvement of cytokine receptors in the action of the toxicant under consideration. This symposium brought together scientists in the area of receptor immunobiology whose expertise in receptor modulation encompassed those major signaling agents involved in the normal immune response, i.e., proinflammatory cytokines, chemokines, interleukins, and interferons. The following is a summary of each of the individual presentations.

In recent years, a great deal of information has been gathered to show that xenobiotics modulate the production of cytokines. However, until recently, the effect of xenobiotics on cytokine receptors has been neglected. The purpose of this symposium was to present information on the effect of several different classes of xenobiotics on various aspects of receptor function (i.e., post-receptor signal transduction of receptor expression) or the involvement of cytokine receptors in the action of the toxicant under consideration.

Receptors for the major cytokine classes (i.e., interleukins, interferons, chemokines, and tumor necrosis factors) share several common features. In all cases, these receptors are transmembrane proteins that contain extracellular domains for specific binding of their target ligands, as well as intracellular regions that regulate initiation of signaling cascades following cytokine binding. Although many cytokine receptors are glycoproteins (i.e., IL-6R, IL-8R, IFN-R), some are not (i.e., IL-1R [types I and II]), TNFαR [types I and II]). The majority of receptors display complete functionality as monomers, although some require formation of dimer/multimer complexes for binding/post-binding signaling events to occur.

Several cytokine receptors have sequence homologies similar to those of receptors for other major biomolecules, including growth factors and immunoglobulins. As such, cytokine receptors have been classified as members of the growth factor or immunoglobulin “superfamilies”; however, not all the receptors within a given cytokine class are necessarily confined to only one classification. For example, although IL-1R is considered a member of the immunoglobulin superfamily, IL-4R is regarded as part of the hematopoietic/growth factor receptor superfamily, and the various forms of IL-6R permit them to be members of both. Interestingly, while this demonstrates that receptors for different cytokines within a given class have divergent classifications, it is also possible for receptors for differing cytokine classes (i.e., IL-4R and IFN-R) to display enough overlapping structural homology that they are designated as belonging to the same superfamily.

The session’s co-chairperson, Dr. Kathleen E. Rodgers of the Livingston Research Institute of the University of Southern California, opened the symposium with a brief overview of our current understanding of how various classes of xenobiotics have been shown to act as immunomodulators. This introduction described how the majority of studies, in trying to determine how agents induce immunomodulation, have focused primarily on the effects upon cytokine production by various immune-system cells. Dr. Rodgers then noted that while such information is critical in assessing the impact of an agent upon immunocompetency at the organismal level, it has been the more recent investigations of the impact of toxicants on immune-cell receptors needed both to bind cytokines and to initiate intracellular signaling pathways which have provided the information necessary for better defining those mechanisms underlying immunotoxicologic effect(s).

As cytokines fall into four major categories, i.e., proinflammatory agents, chemokines, interleukins, and interferons, each
speaker (one for each particular class of cytokine) began their presentation with an overview of both the immunobiology and the workings of associated signal transduction pathways of the particular cytokine receptor(s). Using both data garnered from past studies and their most recent findings, the speakers then presented information regarding: the dependency of select xenobiotics upon proper cytokine-receptor expression/functionality in order to mediate their (immuno)pathologic effects; the type and magnitude of xenobiotic-specific effects (i.e., alterations in receptor expression/binding/turnover, as well as in post-binding sig-naling events) upon receptors in each cytokine class; and, potential mechanisms by which xenobiotic-induced changes in cytokine receptor expression/functionality could potentially give rise to altered host immunocompetence.

Following the final presentation by the session’s other co-chairperson, Dr. Mitchell Cohen, Dr. Rodgers presented the symposium summary. In this presentation, it was made clear that research to specifically examine the effects of toxicants upon cytokine receptors is only now receiving its due attention as an area of vital interest to the field of immunotoxicology. The summary also made it apparent that, due primarily to the limited research on the subject matter, it is not yet possible to make generalized predictions as to the potential magnitude (if any) of damage that varying classes (or specific agents within a given class) of xenobiotics are likely to impart to cytokine receptors; any structure-activity relationship(s) between a type of xenobiotic and an observed effect on cytokine receptor expression/functionality; and, which cytokine receptor class(es) (on each of the myriad immune cell types) might be more/most susceptible to modulation by a given xenobiotic.

The segments below provide, in greater detail, the specifics of each of the presentations.

**Tumor Necrosis Factor α (TNFα)-Mediated Immunotoxicity: Differential Signaling Delineated through TNF Receptor (TNFR) Knockout Mice**

(Lawrence B. Schook)

The proinflammatory cytokine tumor necrosis factor (TNF)α has been implicated as a negative mediator of hepatotoxicity in numerous model systems including carbon tetrachloride, cadmium, dioxin, and acetaminophen. In addition, previous data from our laboratory indicates that TNFα participates in dimethylnitrosamine (DMN)-induced liver injury (Rutherford et al., 1997; Schook et al., 1992), suggesting a role of TNFα in hepatocellular damage initiated during xenobiotic exposure. Therefore, TNFα receptor knockout (TNFR KO) mice lacking either the p55 (TNFR55), the p75 (TNFR75), or both receptors were utilized to (1) examine how TNFα-mediated signals contribute to liver damage during DMN exposure in vivo, and (2) discover the molecular responses associated with each TNFα receptor. Single TNFR KO animals permit linkage of specific events to one receptor or the other during DMN exposure. Liver damage and hepatic cellular infiltration were evaluated microscopically in animals exposed for up to 14 days to DMN. In addition, hepatic gene expression was compared between vehicle- and DMN-treated mice using differential display reverse transcription-polymerase chain reaction (DDRT-PCR) to identify TNFR-regulated genes associated with DMN hepatotoxicity.

Wild type (WT; p55+/−/p75+/−), TNFR55 KO (p55−/−/p75+/−), TNFR75 KO (p55+/−/p75−/−), and TNFR double KO (p55−/−/p75−/−) mice received single daily intraperitoneal (ip) injections of either vehicle (PBS) or DMN (1.5 or 5.0 mg/kg) for up to 14 d. Mice were sacrificed 6 h post-exposure on days 1, 4, 7, 10, and 14 and livers removed to be either fixed in 4% buffered-paraformal-dehyde for histopathological analyses or flash frozen in liquid nitrogen and stored at -80°C for RNA isolation.

Exposure to DMN ip induces a hepatic inflammatory response characterized by T-lymphocyte and neutrophil/macrophage influx accompanied by hepatic cytokine gene expression (Lockwood et al., 1995; Schook et al., 1992). Histopathological analyses were performed to separately describe hepatocellular disruption and infiltration of immunocytes (Fig. 1). TNFR75 KO and double KO mice exhibited a higher degree of centrilobular necrosis by day 4 as compared to TNFR55 KO mice following exposure to DMN (5.0 mg/kg). Following the 7th daily dose of DMN, TNFR55 KO mice had the highest level of necrosis compared to WT, TNFR75 KO, and double KO mice. Throughout the remainder of the DMN-dosing regimen, the level of hepatotoxicity did not vary between WT and all three strains of TNFR KO mice (Fig. 1A). This suggests that TNFα signaling is not required for necrosis during more chronic (days 10–14) exposures, but may be important during early (days 1–7) exposures. In contrast, data showed differential effects of TNFα receptor signaling during DMN-induced cellular infiltration. Following the 7th daily dose of DMN, the TNFR55 KO mice had higher degrees of cellular influx when compared to TNFR75 KO and double KO mice (Horn et al., 1997). On day 14, the levels of infiltrating cells within the liver were equal for all strains of TNFR KO mice, but were still decreased compared to WT controls (Fig. 1B). Thus, these data show that DMN-induced hepatotoxicity can occur in the absence of liver inflammation and that infiltrating cells are not required for hepatotoxicity.

Using DDRT-PCR, we identified several hepatic mRNAs that were differentially expressed in WT and TNFR KO mice as a consequence of DMN-induced hepatotoxicity. These genes have been cloned and their differential expression confirmed by Northern blotting, RNase protection assay, RT-PCR, and/or in-situ hybridization (Bhattacharjee et al., 1997; 1998). Several identified genes showed DNA sequence similarity to previously characterized genes, in addition to identifying novel DNA sequences.

C-Reactive protein (CRP) mRNA was induced in all strains of TNFR KO mice during DMN exposure, but the greatest level of expression was observed in TNFR75 KO and double KO mice (Rutherford et al., 1997). Thus, TNFα signals through TNFR55 to
alter CRP expression. As stated above, the TNFR55 KO mice had the highest level of cellular infiltrates following the seventh daily DMN dose. Xenobiotic exposure may result in CRP production by hepatocytes which results in inhibition of neutrophil infiltration and formation of inflammatory foci.

Using WT mice, we have characterized the expression of cDNAs that were altered following DMN exposure (see Bhat-tacharjee et al., 1998). A cytochrome P450 isoenzyme (cholesterol 7α-hydroxylase), a monokine (a myeloid cell differentiation protein), and mouse urinary protein (MUP) were all suppressed during DMN exposure in vivo. We also observed DMN induction of two cDNAs that are characteristic of an acute phase response; complement factor 3 and serum amyloid A. in-situ hybridization showed that the down-regulation of hepatic MUP mRNA occurred in the centrilobular region of the liver, which is also the primary site where necrosis occurs following DMN exposure. Thus, the utilization of DDRT-PCR has identified several differentially expressed hepatic mRNAs associated with various doses and stages of DMN exposure that are either dependent or independent of TNFα signaling.

In summary, with the utilization of TNFR KO mice, we have applied histopathological, cellular, and molecular techniques to characterize changes that occur within the liver during DMN exposure in order to determine which TNFα signals contribute to specific events during DMN-induced hepatotoxicity. Current studies focus on elucidating the role of novel transcripts identified by DDRT-PCR in both hepatocytes as well as immunocytes associated with DMN exposure and evaluating their potential use as predictive biomarkers.

Interaction of Distamycin Analogs, Opioids, and HIV-1 Envelope Proteins with Chemokine Receptors
(Joost J. Oppenheim)

The primary function of the low-molecular-weight chemokines is to aid in the recruitment, trafficking, proliferation and cytokine expression of leukocytes in response to host infection/injury. Chemokines are classified into 4 groups, based upon the positioning of a cysteine residue within the NH2-terminus of each peptide. These groups include: the C (including lymphotactin); CC or β; including macrophage inflammatory proteins MIP-1α and -β, monocyte chemoattractant proteins MCP-1, -2, and -3, and RANTES); CXC (or α; including IL-8 and platelet factor 4); and CXXXC chemokines, where X indicates intervening amino acid residues. Evidence suggests that CXC chemokines preferentially act upon neutrophils and neutrophil-mediated inflammation, while CC chemokines act on monocytes (but not neutrophils) and tend to be involved in chronic inflammation (Wells et al., 1996); the latter agents also display activities towards basophils, eosinophils, and T-lymphocytes (Bargiolini et al., 1995).

All chemokines bind to receptors (i.e., CR, CCR, CXCR, and CXXXCR) that are members of a G-protein-coupled serpentine receptor superfamily, which spans the leukocyte cell surface membrane 7 times. Most chemokines possess two major binding surfaces, with a high-affinity site responsible for receptor specific-ligand interaction (i.e., CC agents will not bind to CXC receptors and vice versa) and a lower affinity site (i.e., the heparin- or glycosaminoglycan-binding domain), which is thought to help in the establishment and presentation of chemokine gradients on the cell surface and in the extracellular matrix (McFadden and Kelvin, 1997). Within chemokines, critical receptor binding regions are localized to the terminal 10–20 residues of the NH2 terminus (Clark-Lewis et al., 1995). Like most larger cytokines, the majority of chemokines function and bind their receptors as monomers, the primary form in which they exist under physiological conditions; however, dimerization and aggregation do frequently take place.

Distamycin analogs block in vitro and in vivo HIV-1 entry into host cells. In addition, distamycin also blocks the binding...
of chemokines to the chemokine receptors CCR5, CCR3, and CXCR4, but not to CXCR2, and suppresses chemokine-induced intracellular calcium flux and chemotaxis.

The endogenous opioid met-enkephalin inhibited human neutrophil chemotaxis to IL-8 and monocyte chemotaxis to MIP-1α, RANTES, and MCP-1. This response was mediated by μ and δ opiate receptors, and was observed using morphine. Opiates induced phosphorylation of the chemokine receptors CXCR1 and CXCR2, but neither met-enkephalin nor morphine perturbed chemokine receptor internalization. Thus, opiate-induced heterologous desensitization of chemokine receptors may contribute to immunosuppression.

It was also established that HIV-1 envelope proteins interfere with chemokine-chemokine receptor interactions. Preincubation of gp120 from either laboratory-adapted or primary isolates, for 60 min at 37°C, with monocytes, inhibited their capacity to bind chemokines. Similarly, this incubation with gp120 also resulted in a markedly reduced chemotactic response and inducible calcium flux in response to chemokines and fMLP. This heterologous desensitization by gp120 required an interaction with CD4 and resulted in internalization of chemokine receptors. Thus, shed gp120 may participate in viral interference and suppress host defenses by disarming mononuclear cells.

### Suppression of IL-2-Dependent T-Lymphocyte Proliferation by Phenolic Components of Cigarette Smoke (Brian M. Freed)

Although an association between smoking and lung cancer has been evident since the 1930s, only recently have the immunosuppressive effects of smoking been recognized. Pyrolysis of tobacco leaf pigments yields up to 100 μg hydroquinone (HQ), 280 μg catechol, and 70 μg phenol per cigarette. While phenol has no demonstrable effect on T-lymphocytes at concentrations up to 1 mM, HQ and catechol cause an immediate cessation of IL-2-dependent T-lymphocyte proliferation (Li et al., 1996). The inhibitory effect of HQ is entirely distinct from that of the thiol-reactive p-benzoquinone, indicating that the block of T-lymphocyte proliferation is not mediated by its oxidation to a quinone (Geiselhart et al., 1997). The effect of catechol, but not HQ, can be reversed by addition of exogenous iron, suggesting that it inhibits DNA synthesis by chelating iron (Li et al., 1997).

One of the earliest events induced by IL-2 is the expression of transferrin receptors (TfR, CD71), which promotes the uptake of extracellular iron. HQ inhibits TfR expression on HTL to a level comparable to IL-2-starvation, but neither catechol nor phenol inhibits TfR expression. However, the inhibition of TfR expression by HQ occurs only after 24 h, while inhibition of DNA synthesis is immediate. This observation suggests that HQ has a separate effect on DNA synthesis.

Short-term exposure of human T-lymphocytes to HQ resulted in a preferential suppression of DNA synthesis over RNA synthesis, indicating that HQ might interfere with ribonucleotide reductase, a rate-limiting, iron-dependent enzyme that catalyzes the reduction of ribonucleotides to deoxyribonucleotides. This hypothesis is supported by the observation that a combination of AdR and GdR reduces the inhibitory effects of HQ by 37%, while the corresponding ribonucleotides have no effect. AdR and GdR also partially reverses the effects of catechol, which is consistent with the role of iron in ribonucleotide reductase activity (Li et al., 1997).

The Jurkat T-lymphocyte line, stably-transfected with the M2 subunit of ribonucleotide reductase, exhibited a 3-fold increased resistance to HQ, while Jurkat T-lymphocytes transfected with the M2 gene in the reverse (nonsense) orientation exhibited the same sensitivity to HQ as the nontransfected control (Li et al., 1998). In addition, induction of hydroxyurea resistance in Jurkat cells by continuous cultivation in progressively higher concentrations of the drug concomitantly rendered the cells resistant to HQ. Taken together, these experiments provide strong evidence that HQ, like hydroxyurea, blocks T-lymphocyte proliferation by inhibiting ribonucleotide reductase.

The typical cigarette delivers ~100 μg of HQ to the lungs, which is sufficient to generate 18 ml of a 50 μM solution. This high concentration of HQ may provide a selective advantage to the growth of tumor cells by preferentially suppressing lymphocyte proliferation. The growth of carcinoma of the lung, and solid tumors in general, is highly resistant to inhibitors of ribonucleotide reductase. In addition, transformed cells readily develop resistance to inhibitors of ribonucleotide reductase by overexpressing the M2 subunit. Finally, HQ promotes the metastasis of lung carcinoma cells (Gopalakrishna et al., 1994). Thus, the presence of HQ may have little or no deleterious effect on the growth of the tumor, while completely abrogating the T-lymphocyte response. A summary of the effects of the phenolic components of cigarette tar is shown in Table 1.

<table>
<thead>
<tr>
<th>Inhibits DNA synthesis (IC_{50})</th>
<th>Reversible by FeCl(_3)</th>
<th>Blocks TfR expression</th>
<th>Reversible by transfection with RNR-M2</th>
</tr>
</thead>
<tbody>
<tr>
<td>HQ</td>
<td>6–10 μM</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Catechol</td>
<td>6–10 μM</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Phenol</td>
<td>&gt;1 mM</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

### Alterations in Interferon (IFN) Receptor Binding/Post-Binding Events Induced by Xenobiotics (Dr. Mitchell D. Cohen)

The interferons (IFN) represent a class of cytokines that utilize signal transduction pathways to activate specific genomic regions (such as the IFNα/β-induced ISRE [interferon-sensitive response element] and IFNγ-induced GAS [gam-
ma(IFN)-activated site] within the cell nucleus (Briscoe et al., 1996; Pestka, 1997). All nucleated cells and erythrocytes have IFN receptors (IFN-R) distributed either in horizontal patches, vertical pillars, or randomly over their surfaces.

There are two possible mechanisms to describe how IFN might trigger activation of the specific nuclear response element after receptor binding. In one mechanism, the IFN—IFN-R complex is internalized into receptosomes that transverse the cytoplasm. The complex begins to dissociate within this organelle; while most liberated IFN is degraded, some leaves the receptosome and crosses into the nucleus to bind to ISRE/GAS sites. In this mechanism, some IFN-R is salvaged and eventually returned intact to the cell surface.

In a second mechanism, IFN binding to IFN-R triggers a sequence of (de)phosphorylative events which culminate in ISRE/GAS activation. Binding of IFNα/β to IFNα/β-R activates the Janus kinases (i.e., Jak1 and Tyk2) associated with the cytoplasmic portion of each unit of the receptor dimer, which then phosphorylate three nascent cytoplasmic STAT (signal transducer and activator of transcription) proteins, p91/STAT1α, p84/STAT1β, and p113/STAT2, that associate to form an ISGF3 complex that crosses into the nucleus. This complex then unites with DNA-binding p48/ISGF3γ to form the mature ISRE-binding ISGF3 complex.

Conversely, IFNγ binding to the IFNγ-R dimer causes two additional receptor proteins to complex with the binding dimer. Each arm of the final tetramer has a Janus kinase associated with its cytoplasmic element (binding dimer proteins contain Jak1; outer proteins contain Jak2) which cross-activate one another and subsequently phosphorylate cytoplasmic STAT1α proteins. As monomeric pp91 or dimeric pp91-pp91, the now-activated GAF [gamma-activating factor] crosses into the nucleus and binds to GAS regions of the DNA.

In this second mechanism, it is thought that surface IFN—IFN-R complexes are internalized and degraded within lysosomes rather than being recirculated from receptosomes. To assure that some surface IFN-R expression always occurs (the amount of which is subject to up- or down-regulation in response to the specific IFN itself), IFN-R are either released pre-formed from cellular pools or are synthesized de novo as part of the IFN-induced response.

Because numerous steps are required to achieve cell activation with IFN, xenobiotics might impact upon this process at the level of: (1) the IFN ligand; (2) IFN-R expression; (3) IFN-R affinity; (4) IFN—IFN-R complex internalization and/or intracellular processing; (5) GAF/ISGF3 complex formation/dissociation and/or Jak/STAT (de)activation; and/or, (6) GAF/ISGF3 complexation with GAS/ISRE sites. Indeed, it has been shown that many classes of xenobiotics (Table 2) act at one or more of these levels to alter cell responses to IFNs.

Among agents that down-regulate cell responses to IFNs, transforming (TGF-α) and epidermal (EGF) growth factors (Mitra and Nickoloff, 1992), as well as arachidonic acid and its metabolites (Menon et al., 1990), cause decreased IFN-R expression without changing receptor affinity. Conversely, suramin changes IFN-R affinity without affecting expression (Kanderer-Szerszen et al., 1997). Lastly, heparin and heparin sulfate decrease cell responses to IFNs without causing changes in either IFN-R expression or affinity but, rather, by interfering with the electrostatic interactions of IFN with the IFN-R (Douglas et al., 1997).

Among microbial agents known to down-regulate cell responses to IFN, Listeria monocytogenes (but not L. innocua) inhibits inducible IFN-R expression in infected bone marrow-derived macrophages (Demuth et al., 1996) but not infected macrophage-like cell lines. Trypanosoma cruzi reduces IFN-R expression on infected lymphocytes, especially B-lymphocytes (Kierszenbaum, et al., 1995). Conversely, Leishmania donovani appears to alter only post-binding events so as to block expression of ISRE/GAS-induced gene products in the infected cell (Reiner et al., 1988). For all three organisms, the mechanism(s) for these effects are unknown.

Some commonly-encountered environmental/occupational metals also down-regulate cellular IFN-inducible responses. Vanadium, through changes in intracellular signaling or enhanced expression of inactive IFN-R, reduces IFN-R expression while simultaneously increasing the affinity of those that remain (Fig. 2) (Cohen et al., 1996). This unusual increase in IFN-R affinity may result from enhanced salt bridging between IFN and carboxylate groups in receptor acidic amino acids. Unlike vanadium, lead appears to affect neither IFN-R binding nor expression. Rather, lead decreases the internalization/intracellular processing of IFN—IFN-R complexes in exposed cells (Mauel et al., 1989).

Among agents which affect post-binding events, both UV light and phorbol esters (i.e., PMA), perhaps through phospha-

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**TABLE 2**

<table>
<thead>
<tr>
<th>Agents That Have Been Shown to Modify IFN-Induced Responses in Cells</th>
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<tbody>
<tr>
<td><strong>Up-regulating Agents</strong></td>
</tr>
<tr>
<td>Cytokines (IL-1, TNFα)</td>
</tr>
<tr>
<td>Colony-stimulating factor (GM-CSF)</td>
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<tr>
<td>Prolactin</td>
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<tr>
<td>Tamoxifen/medroxyprogesterone/mifepristone</td>
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<tr>
<td>Difluoromethylornithine</td>
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<tr>
<td>Hydroxyurea</td>
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<tr>
<td>Mezeiren</td>
</tr>
<tr>
<td>DMSO</td>
</tr>
<tr>
<td>Sodium butyrate</td>
</tr>
<tr>
<td>Hexamethylene bisacetamide</td>
</tr>
<tr>
<td>Lanthanum (La)</td>
</tr>
<tr>
<td>Ytterbium (Yb)</td>
</tr>
<tr>
<td>LPS</td>
</tr>
<tr>
<td><strong>Listeria monocytogenes</strong></td>
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</tbody>
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tase activation, alter the phosphorylation of STAT proteins needed for GAS/ISRE activation (Aragane et al., 1997; Petricoin et al., 1996). While the effect from UV is specific against STAT1 in cells treated with either IFNα/b or IFNγ, that of PMA is against both STAT1 and STAT2 and only in cells stimulated with IFNα/b.

There are also several agents that act to up-regulate cell responses to IFN. Some cytokines, such as tumor necrosis factor-α (TNFα) and interleukin-1α (IL-1α), as well as lipopolysaccharide (through induced TNFα and IL-1α), cause increased IFN-R expression on treated cells (Krakauer and Oppenheim, 1993). Similarly, anti-cancer agents like hydroxyurea (HU), difluoromethylornithine (DFMO), and mezerein increase cell responses to IFN, in part, by inducing increased IFN-R expression (Dilollo et al., 1990; Tamura et al., 1997). The effects of HU may be mediated via Jak1/Tyk2, then STAT1 activation, while DFMO seems to inhibit ornithine decarboxylase to reduce cell polyamine levels to below those which cause down-regulation of IFN-R expression. Mezerein induces differentiation that, in turn, stimulates IFN-R expression. Conversely, other differentiation-inducing agents, including dimethyl sulfoxide, sodium butyrate, sodium phenylacetate, and hexamethylene bisacetamide, also increase cell responses to IFN (Goto et al., 1996), but apparently not via altered IFN-R expression or affinity.

Finally, in one study, modification of IFNβ with lanthanum or ytterbium metals led to binding which could only be competed against with excess cold metal-treated IFNβ (Sedmak et al., 1986). This indicated that by altering the ligand itself, cellular responses to IFNβ might be increased through heretofore unexpected interactions, possibly even at non-IFNβ-R/IFNβ-R-like sites on the cell surface.

In summary, xenobiotics can affect the interactions of cells with IFNs (α, β, or γ type) to either augment or inhibit the cell response to IFNs. Six possible sites of action, each of which has been shown to be altered by xenobiotics, were reviewed. It is clear that although xenobiotics have a capacity to affect interactions of cells with the differing classes of IFN, it is not yet possible to predict how a specific member of any one agent interacts with IFN-R class I and IFN-R class II receptors, respectively.

FIG. 2. Effect of vanadium exposure upon macrophage surface IFNγ receptor binding activity and expression. Scatchard analyses of the specific binding (22°C, 4 h) of 125I-IFNγ to WEHI-3 mouse macrophages pretreated 20 hr at 37°C with (A) RPMI 1640 medium only or (B) 100 μM ammonium metavanadate. Each curve is a construct using 10–15 IFNγ concentrations (i.e., 5 pM–1 nM) per assay; for ease of visualization, only data points for the B (bound ligand) and B/F (bound/free ligand) values obtained with 50, 100, 200, 500, and 1000 pm IFNγ are shown. Resultant best-fit lines for determining Kd and expression values for each IFNγR class are indicated using either open stars/X symbols or diamond shapes for IFNγR Class I and IFNγR Class II receptors, respectively.
class (i.e., metal, pharmacologic, microorganism, or cytokine) will act.

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