Interleukin 24 (MDA-7/MOB-5) Signals through Two Heterodimeric Receptors, IL-22R1/IL-20R2 and IL-20R1/IL-20R2* 

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Interleukin 24 (IL-24) encodes a secreted protein that exhibits significant homology to the interleukin 10 (IL-10) family of cytokines. Here we show that the human IL-24 is secreted by activated peripheral blood mononuclear cells and is the ligand for two heterodimeric receptors, IL-22R1/IL-20R2 and IL-20R1/IL-20R2. The latter is also the receptor for IL-20. COS cells transfected with either IL-24 receptor heterodimers bind the ligand with similar saturation kinetics. IL-24 binding to either its endogenous receptors on human keratinocytes or to ectopically expressed receptors on baby hamster kidney cells leads to activation of the signal transducers and activators of transcription. Taken together, these results provide compelling evidence for IL-24 being the fourth member of IL-10 family of cytokines to which their specific receptors have been identified.

Interleukin 10 (IL-10)§ was discovered over a decade ago as a potent immunomodulator capable of eliciting a wide range of cellular effects which include inhibition of proinflammatory cytokine production (for review, see Ref. 1). However, it was not until recently, 5 more cellular genes, encoding secreted proteins with 20–30% homology to IL-10, were described. They are IL-19 (2); IL-20 (3); IL-22, originally designated as IL-TIF (4–5); AK155 (6) and mda-7 (7), which has been renamed as IL-24 with the approval of the HUGO Gene Nomenclature Committee. The human IL-24 (mda-7) was originally identified as a gene induced in certain melanoma cells by treatment with interferon-β and mezerein, which caused terminal differentiation of cells through poorly understood mechanisms (7). Rat ortholog of human IL-24 was later identified and designated as either c49a, a gene induced during wound healing (8), or mob-5, a gene induced by ras oncopogenes (9). Mouse IL-24 (FISP) was also recently identified and shown as an IL-4-induced protein secreted by Th2 cells (10). The proof that IL-20 and IL-22 are indeed members of the IL-10 family of cytokines came when their functional cell surface receptors were identified and characterized (3, 11–12). While IL-22 is linked to acute-phase protein production in hepatoma cells and in inflammation (5), the biological functions of IL-20 appear to be involved in epidermal development (3).

The IL-10 receptor was initially identified as a single R1 type of receptor with a long cytoplasmic domain, IL-10R1 (13). Later it was found that the functional IL-10 receptor required a second chain of a R2 type of receptor with a short cytoplasmic domain (IL-10R2) (14). Like IL-10, both IL-20 (3) and IL-22 (11–12) bind to and signal through heterodimeric receptors each with a R1 and a R2 type of receptor subunit. Both subunits of the IL-20 receptor were initially discovered as orphan receptors in the class II cytokine receptor family, similar to those of IL-10 (3). However, the IL-22 receptor consists of a novel R1 type of class II cytokine receptor and the previously known IL-10R2 (11, 12). Although IL-10 and IL-22 share the same R2 receptor subunit, the receptor activation is ligand-specific (11, 12). Binding of these cytokines to their corresponding receptors leads to the activation of JAK-STAT pathways (for review, see Ref. 15).

As a result of our ongoing efforts to identify genes involved in ras oncopgene-mediated cell transformation by a differential display strategy (16–20), we have identified the rat IL-24 (mob-5) as an oncogenic Ras-specific target gene, which, like its human ortholog, encodes a secreted protein with 23% homology to IL-10 (9). We have provided evidence showing that IL-24, which is induced by oncogenic ras through the mitogen-activated protein kinase pathway, may function through its cell surface receptor(s) in either an autocrine or paracrine fashion in the course of neoplastic transformation (9). Several lines of evidence also support a potential involvement of the IL-10 family of cytokines in cell transformation and cancer development. It has been shown that many DNA viruses, including the DNA tumor virus Epstein-Barr virus, cytomegalovirus, Yaba-like disease virus, and herpesvirus saimiri either encode their own IL-10-like proteins (21–23), or are able to induce cellular proteins with homology to IL-10 upon viral infection (6). Although exhibiting rather restricted tissue expression, IL-22R1 is found to be expressed by a variety of cell lines derived from solid tumors (11).

To provide insight into the biochemical and biological functions of IL-24 in normal development as well as during cell transformation and tumorigenesis, we report here the identification and characterization of its functional cell surface receptors. The novel scheme of receptor subunit swapping uncovered in this study provides a molecular basis for ligand cross-talk among members of IL-10 family of cytokines.

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¶ The abbreviations used are: IL-10, interleukin 10; IL-20, interleukin 20; IL-22, interleukin 22; IL-24, interleukin 24; LPS, bacterial lipopolysaccharide; ConA, concanavalin A; PBMCs, peripheral blood mononuclear cells; TNF, tumor necrosis factor; ELISA, enzyme-linked immunosorbent assay; STAT, signal transducers and activators of transcription; BHK, baby hamster kidney.

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MATERIALS AND METHODS

**Cell Lines and Culture**—All cell lines including 293T (GenHunter Corp., Nashville, TN), HaCaT (3), and BHK (from ATCC) were grown in Dulbecco’s modified Eagle’s medium (Invitrogen, Grand Island, NY) with 10% fetal bovine serum (HyClone, Logan, UT) and 1% penicillin-streptomycin (Invitrogen) at 37°C with 10% CO2. COS-E5 (GenHunter Corp.), a clonally purified COS-1 cell line, was maintained in Dulbecco’s modified Eagle’s medium with 10% bovine calf serum and 1% penicillin-streptomycin.

**RNA Purification, Reverse Transcribe-PCR, and Southern Blot Hybridization**—Total RNA from cultured cells was purified using the RNPure reagent following the manufacturers instructions (GenHunter Corp.). The cDNA synthesis, reverse transcriptase-PCR, and Southern blot hybridization were carried out as described previously (9).

**Production of Secreted Human Placental Alkaline Phosphatase (AP), IL-24-AP Fusion Proteins and His-tagged IL-24 (IL-24-His)**—Production of secreted AP and IL-24-AP fusion proteins were described previously (9, 24). For the production of human IL-24-His, the PCR primer was used to attach 9XHis to the C terminus of IL-24, and the resulting PCR product was subcloned into the BglII site of the pAPtag-5 expression vector (GenHunter Corp.). After transfection into the 293T cells, stable cell lines constitutively secreting IL-24-His into the conditioned media were obtained.

**Receptor Expression Constructs and Cell Transfection**—The coding regions of human IL-10R2 (14), IL-20R1 (3), IL-20R2 (3), and IL-22-R1 (11) were PCR amplified and subcloned into the KpnI and XbaI sites of the expression vector pcDEF3. The complete coding region of human IL-20R1 was also PCR amplified using an EST clone (IMAGE number 7342070) (ATCC, Manassas, VA) as a template, subcloned into the BglII site of the pAPtag-5 expression vector (GenHunter Corp.). The complete coding region of human IL-20R1 was also PCR amplified using an EST clone (IMAGE number 7342070) (ATCC, Manassas, VA) as a template, subcloned into the BglII site of the pAPtag-5 expression vector (GenHunter Corp.). The cDNA synthesis, reverse transcriptase-PCR, and Southern blot hybridization were carried out as described previously (9).

**Quantitative Real-Time PCR**—Total RNA from cultured cells was purified using RNPure reagent following the manufacturers instructions (GenHunter Corp.). The cDNA synthesis, reverse transcriptase-PCR, and Southern blot hybridization were carried out as described previously (9).

**Induction of IL-24 by activated PBMCs.** Freshly prepared human PBMCs were treated with PBS, LPS, or ConA for 2 and 4 h. The resulting conditioned media were subsequently analyzed by Western blot analysis using monoclonal antibody specific to human IL-24, which showed that ConA, but not LPS, was able to cause a rapid induction of IL-24. The result shown was representative of at least three independent experiments. PBS, phosphate-buffered saline.

**Western Blot Analysis**—The proteins in conditioned media were separated by SDS-PAGE, transferred to a nitrocellulose membrane, and probed with primary antibodies followed by secondary antibodies. The immunoblots were visualized by chemiluminescence and quantified using ImageJ software (NIH, Bethesda, MD).

**Glycosylation of human IL-24.** A, conditioned media of 293T and 293T stably transfected with the IL-24-His expression vector were analyzed by Western blot using IL-24 specific antibody. B, glycosylation of the human IL-24. The partially purified IL-24-His before (control) and after digestion with endo-β-N-acetylglucosaminidase (PNGase F) alone, or PNGase F plus endo-O-glycosidase, were analyzed by Western blots using an monoclonal antibody specific to IL-24. The result shown was representative of at least two independent experiments.

**Hybridization—Penicillin-streptomycin.** Hybridization—Penicillin-streptomycin.

**Deglycosylation**—Deglycosylation of human IL-24-His was partially purified from the conditioned medium using Ni-NTA beads (Qiagen), following the manufacturers instructions. Deglycosylation of N- and O-linked carbohydrates from denatured IL-24-His was carried out with endoglycanase F (PNGase F) and endo-O-glycosidase (Prozyme, San Leandro, CA, respectively, following the manufacturers recommended procedure (26). Deglycosylation of human IL-24-His was purified from denatured human IL-24-His by using human IL-24-AP as an antigen as well as a reporter. Mice were then boosted with an additional 25–100 μg of purified human IL-24-AP fusion protein to enhance the production of antibodies recognizing native, rather than, denatured IL-24. Three days prior to fusion, mice were hyperimmunized, again with 25–100 μg of IL-24-AP. Spleens from hyperimmunized mice were then fused with SP2/O myeloma cells and hybridomas producing anti-Mob5-specific hybridomas were subcloned according to standard procedures.

**Purification of PBMCs and Induction of IL-24 by Concanavalin A (ConA)**—Human PBMCs were freshly purified from healthy donors by gradient centrifugation using LSM lymphocyte separation medium (ICN, Costa Mesa, CA) according to the manufacturers instructions. After resuspension in 150 μl of RPMI medium with 10% fetal bovine serum at a density of 10^5 cells/well in a 96-well plate, the cells were stimulated with either 100 ng/ml bacterial lipopolysaccharide (LPS) (Sigma) or 25 μg/ml ConA (Sigma) for 2 and 4 h. The conditioned media with or without stimulation were analyzed for IL-24 production by Western blot analysis described as described (9), using a monoclonal antibody to human IL-24 (clone L14).

**Gel Shift Assay**—Two days after transfection as described above, cells were exposed to 1 ml of Dulbecco’s modified Eagle’s medium containing 0.1 volume of conditioned medium from 293T cells without or with IL-24-His (final concentration at 50 ng/ml). Thirty minutes following the treatment, nuclear extracts were prepared by the standard protocol. For STAT-specific gel shift assays, the STAT-specific double stranded DNA probe GRR (5’-ATGATTTCTCCAGAAA-3’/5’-CCCTTCTCGGAAATAC-3’) was end labeled with ^32P using Klenow (Invitrogen), incubated with the nuclear extracts and separated on a 5%–10% nondenaturing polyacrylamide gels as described previously (9).

**Supershift experiments, 1 μg of STAT-1 or STAT-3 specific antibody was used (Santa Cruz Biotechnology Inc., Santa Cruz, CA).**

**LPS-induced TNF-a Production from PBMCs**—Freshly prepared PBMCs (see above) were resuspended at a density of 10^6 cells/well in 150 μl of RPMI plus 10% fetal bovine serum in a 96-well plate. LPS was then added to each well at a final concentration of 80 ng/ml in the absence or presence of either 20 ng/ml recombinant human IL-10 (R&D Systems, Minneapolis, MN) or 100 ng/ml human IL-24-His, or both, for 5 h. TNF-a levels in the conditioned media of the cells were determined with an ELISA kit for human TNF-a (R&D Systems, Minneapolis, MN) as instructed by the manufacturer. Each data point was determined in duplicate.
RESULTS

Induction of IL-24 by Activated PBMCs—Although we have shown previously that IL-24 expression in rodents was not detectable in any normal adult tissues or embryos (9), it was reported by others that the gene appeared to be induced under pathological conditions such as wound healing (8). Given the fact that IL-24 belongs to the IL-10 family of cytokines which can be induced by ConA-activated T cells (12, 27), we looked at IL-24 induction under similar conditions. Freshly prepared human PBMCs were stimulated with either bacterial LPS or ConA. Following stimulation, the production of IL-24 by PBMCs in the conditioned media was analyzed by Western blot (Fig. 1). ConA induced a rapid secretion of IL-24 by the PBMCs. LPS, on the other hand, had little effect on IL-24 production.

IL-24 Is Glycosylated—The predicted molecular weight of human IL-24 is 23,800, but both the IL-24 secreted by human PBMCs (Fig. 1) and the IL-24-His secreted by 293T stable transfectants appeared to be much bigger, with an apparent molecular weight closer to 35,000 (Fig. 2). To determine whether this is due to glycosylation, partially purified IL-24-His was subjected to digestion with either endoglycanase F (PNGase F) which is specific for asparagine-linked (N-linked) carbohydrates, or PNGase F plus endo-O-glycosidase, which is specific for serine/threonine-linked (O-linked) oligosaccharides. As predicted, PNGase F converted the 35-kDa form of IL-24-His to a single band of lower molecular mass of about 23 kDa (Fig. 2B). It appeared that the extensive glycosylation of human IL-24 was all N-linked type, since endo-O-glycosidase treatment had little effect on the protein.

Identification of the IL-24 Receptors—Since IL-24 shares significant homology to IL-10, we predicted that IL-24 is a member of the IL-10 family of cytokines (9). Supporting this hypothesis was our finding that not only IL-24, but also its putative cell surface receptor, appeared to be induced by ras oncogenes (9). Shortly after our initial description of IL-24, two more members of IL-10 family of cytokines, IL-20 and IL-22, and their receptors were reported (3, 11). Sequence alignment indicated that IL-24 and the other members of the IL-10 family of cytokines share an overall homology ranging from 24 to 33%. Among these, IL-24 is closest to IL-20 with 33% homology over all, and the highest homology is found at the C termini of the proteins.

Based on published data and data base searches of GenBankTM (NCBI), only three R1 and two R2 types of the IL-10 family of receptor subunits can be identified. The three R1 subunits are IL-10R1, IL-20R1, and IL-22R1, whereas the two R2 subunits are IL-20R2 and IL-10R2, the latter of which is also the second receptor subunit for IL-22. Thus we speculated...
that the IL-24 receptor could be a heterodimer of the known R1 and R2 receptor subunits. To determine whether IL-24 is the ligand for any of the heterodimers of known R1 and R2 receptor subunits, we carried out receptor binding assays using either secreted human placental AP or the IL-24-AP fusion protein as a probe (Fig. 3A) (9). The quantitative cell surface binding assays were carried out with COS cells transfected with plasmids expressing IL-10R2, IL-20R1, IL-22R1, and IL-20R2 individually or with all four R1/R2 combinations (Fig. 3B). IL-24-AP, but not AP, exhibited significant binding to COS cells transfected with IL-20R2 alone, and the binding was further dramatically increased when IL-20R2 was co-transfected with either IL-20R1 or IL-22R1. Neither IL-20-R1 nor IL-22R1 alone were able to bind to IL-24-AP. Using AP staining assays, we were able to confirm the quantitative IL-24-AP binding data (Fig. 3C). Specific cell surface staining was detected with IL-24-AP when COS cells were transiently transfected with plasmids expressing IL-10R2, IL-20R1, IL-22R1, and IL-20R2 individually or with all four R1/R2 combinations (Fig. 3B). IL-24-AP, but not AP, exhibited significant binding to COS cells transfected with IL-20R2 alone, and the binding was further dramatically increased when IL-20R2 was co-transfected with either IL-20R1 or IL-22R1. Neither IL-20-R1 nor IL-22R1 alone were able to bind to IL-24-AP. Using AP staining assays, we were able to confirm the quantitative IL-24-AP binding data (Fig. 3C). Specific cell surface staining was detected with IL-24-AP when COS cells were transiently transfected with IL-20R2, but not IL-20R1 or IL-22R1 alone (Fig. 3C). When cells were transiently transfected with IL-20R2 in combination with either IL-20R1 or IL-22R1, the cell surface binding of IL-24-AP was greatly increased (Fig. 3C). In addition to increased ability to bind IL-24-AP, the presence of either R1 subunit resulted in a much more uniformed distribution of the heterodimeric receptor over the entire cell surface (Fig. 3C).

Binding kinetics of IL-24 to its receptors was determined using COS cells transiently transfected with either IL-20R1/IL-20R2 or IL-22R1/IL-20R2 heterodimeric receptors (Fig. 4). IL-24-AP was shown to bind to both receptors with similar affinity and saturation kinetics. The affinity ($K_d$) of IL-24-AP to both IL-20R1/IL-20R2 and IL-22R1/IL-20R2 heterodimeric receptors were about 8 nm.

**IL-24 Signals through Both the IL-20 Receptor (IL-20R1/IL-20R2) and IL-22R1/IL-20R2 Heterodimers**—To functionally determine whether IL-24 can signal through these putative receptors by activating the downstream JAK-STAT pathways, as has been observed for other members of the IL-10 family of cytokines, we performed the following three experiments. First, we examined the expression of IL-22R1 in a human keratinocyte cell line, HaCaT, which was shown to be positive for IL-20 receptor (IL-20R1/IL-20R2) (3). The finding of the coexpression of IL-22R1 and IL-20R2 in the HaCaT, but not the BHK cell line (Fig. 5A), suggests that the former might contain both receptors that are capable of binding IL-24. The gel shift assays were then conducted for the functional analysis of STAT activation in the HaCaT cells by IL-24-His (Fig. 5B). After a 30-min stimulation, IL-24-His-treated cells showed a marked increase in STAT activation (Fig. 5B).

It is possible that the IL-24-induced STAT activation in HaCaT cells could be due to IL-24 binding to either the IL-20 receptor or to the IL-22R1/IL-20R2 heterodimer. To differentiate between these two possibilities, vectors expressing either IL-20R1 and IL-20R2, or IL-22R1 and IL-20R2 were trans-
were determined by ELISA. Unlike IL-10, IL-24 did not appear to shift assay for STAT activation using a 32P-labeled STAT-specific probe. BHK cells, and then processed for gel-shift assay for STAT activation using a 32P-labeled STAT-specific probe (GRR). The results shown are representative of at least four independent experiments.

Disruption of IL-24 receptors by interfering with STAT activation in HaCaT cells. Unlike HaCaT cells, BHK cells do not express either receptor subunit. B, IL-24-dependent STAT activation in HaCaT cells. HaCaT cells were stimulated for 30 min with either 293T control medium or IL-24-His conditioned medium, and then processed for gel-shift assay for STAT activation using a 32P-labeled STAT-specific probe (GRR). The results shown are representative of at least four independent experiments.

**DISCUSSION**

In this report, we show that human IL-24 (MDA-7), which is heavily glycosylated, is produced by PBMCs following ConA stimulation, like most of the members of the IL-10 family of cytokines. While this work was in progress, a gene designated FISP was reported to be a secreted protein produced by murine Th2 cells (10). Based on the sequence homology, FISP is likely the mouse ortholog of IL-24. Together these evidence support that IL-24 is a T cell-derived cytokine. Like IL-22 (12), IL-24 is unable to inhibit LPS induced cytokine production by PBMCs, an activity that still appears to be unique to IL-10 (27, 28). Consistent with this finding, ligand binding studies using AP alone and IL-24-AP, indicated that few cells in PBMCs appeared to have IL-24 receptors (data not shown).

The identification of two functional IL-24 receptor heterodimers, one consisting of two recently identified class II cytokine receptors, IL-22R1 and IL-20R2, and the other being IL-20R1 (IL-20R1/IL-20R2), provides the final proof that IL-24 is indeed a new member of the IL-10 family of cytokines, following IL-20 and IL-22. However, the finding of two different IL-24 receptors with similar affinity to IL-24 raises an interesting issue for future research into whether these are functionally redundant receptors or whether they are differentially used by IL-24, depending on cellular context. Likewise, the utilization of IL-20R1/IL-20R2 by either IL-20 (K_d = 1.5 nM) (3) or IL-24 (K_d = 8 nM) raises the issue as to whether these cytokines induce identical signals downstream or they would compete for utilization of the same receptor to a different biological end point. It is interesting to note that the affinity of IL-10 to its receptor appears to be an order of magnitude higher than those of IL-20 and IL-24 (28). Although the three previously known receptors of the IL-10 family of cytokines are all heterodimeric, each being made up of a R1 type and a R2 type of receptor subunit, IL-10 and IL-22 share the same R2 type of receptor subunit, IL-10R2. It is very

**FIG. 5.** The IL-24 specific activation of STATs. A, Northern blot analysis showed positive for IL-22R1/IL-20R2 receptor expression in HaCaT human keratinocytes. Unlike HaCaT cells, BHK cells do not express either receptor subunit. B, IL-24-dependent STAT activation in HaCaT cells. HaCaT cells were stimulated for 30 min with either 293T control medium or IL-24-His conditioned medium, and then processed for gel-shift assay for STAT activation using a 32P-labeled STAT-specific probe (GRR). The results shown are representative of at least four independent experiments.

**FIG. 6.** IL-24 signals through both heterodimeric receptors of IL-20 (IL-20R1/IL-20R2) and IL-22R1/IL-20R2 by activating STAT-1 and STAT-3. A, BHK cells were transiently transfected with the receptor pairs as indicated. After stimulating the cells with either the control 293T medium or 293T/IL-24-His medium for 30 min, STAT activation was analyzed by a 6% polyacrylamide gel with nuclear extracts from the cells using the 32P-labeled GRR probe. IL-24-His was able to confer specific activation of STATs through both IL-20R1/IL-20R2 and IL-22R1/IL-20R2 heterodimeric receptors. B, IL-24 signals through its receptors by activating both STAT-1 and -3. BHK cells transiently transfected with IL-22R1/IL-20R2 expression vectors were stimulated with either the 293T control medium or IL-24-His/293T medium for 30 min. Gel shift assays were carried out with nuclear extracts using the 32P-labeled GRR probe in the absence or presence of antibodies against STAT-1 and -3 as indicated. A 5% polyacrylamide gel was used for better separation of different forms of STAT-GRR complexes. Both STAT-1 and STAT-3 antibodies caused either disruption (STAT-1) or supershift (STAT-3, indicated by arrowhead) of STAT-GRR complexes induced by IL-24-His, respectively. The results shown were representative of at least two independent experiments.
indicated. TNF-α presence of IL-10 (20 ng/ml), IL-24-His (100 ng/ml), or both cytokines as indicated. TNF-α secreted into the media was quantified with ELISA. Unlike IL-10, IL-24-His had little inhibitory effect on LPS-induced TNF-α production by PBMCs. The error bar represented the standard deviation of duplicate data obtained for each data point within the same experiment. The result shown are representative of at least four independent experiments.

It is interesting to note that IL-24 not only shares the same R2 type of receptor subunit with IL-20, but also its R1 type of receptor subunit is shared with IL-22. The implication from the discovery of such a scheme of receptor subunit swapping is important in two aspects. First, it implies that there could be a total of six combinations of heterodimeric receptors made up of three known R1 and two known R2 receptor subunits, to which four combinations now have already assigned ligands, IL-10, IL-20, IL-22, and IL-24 (Table 1). Thus, two more receptors made up of IL-10R1/IL-20R2 and IL-20R1/IL-10R2 may also have their specific ligands. Incidentally, there are indeed two more IL-10-like cytokines without their known receptors, IL-19 (2) and AK155 (6), both of which are encoded by cellular genes. It is possible that more R1 or R2 receptor subunits remain to be discovered and the discovery of the receptor-ligand relationship presented in this study should be helpful for the identification and assignment of their corresponding ligands. Obviously, the current nomenclature for the IL-10 family of receptor subunits has not taken the consideration of such extensive receptor subunit swapping. The use of the CRF2-X designation system (15) may turned out to be more appropriate, since any given R1 or R2 receptor subunit may be shared by more than one ligand.

Second, the scheme of receptor subunit swapping uncovered here further supports the prediction that there is cross-talk among the IL-10 family of cytokines (11). Clearly shown by this study, like IL-20 (3), IL-24 can signal through the IL-20 receptor. Conversely, IL-20 may also be able to signal through the new IL-24 receptor (IL-22R1/IL-20R2). Moreover, our finding of IL-22R1 being the first R1 type of receptor subunit shared by two different ligands of the IL-10 family of cytokines, would also predict a potential cross-talk between IL-22 and IL-24. For example, although we showed that IL-24 could not signal through the IL-22 receptor (IL-22R1/IL-10R2), it is not clear if IL-22 will be able to signal through the IL-24 receptor(s). Such potential cross-talk among the IL-10 family of cytokines and their receptors can now be readily tested.

Dysfunction in the control of such ligand-receptor cross-talk as a result of aberrant expression of the cytokines and their receptors could be one of the underlying causes for diseases, such as cancer. We have shown that ras oncogenes cause constitutive expression of IL-24 in cells from nonhematopoietic origin (9). Ras transformed rat embryo fibroblasts and intestinal epithelial cells not only constitutively produce IL-24, but also appear to have increased expression of the IL-24 receptor(s) (9). Like IL-20, one of the likely normal target tissues for IL-24 is skin, since the human keratinocyte cell line HaCaT appears to express both endogenous IL-24 receptors. This is also consistent with the finding that IL-24 is transiently expressed in lesions of skin injury from rat wound healing experiment (8). While little expression of IL-22R1 was detected from colon and lung, overexpression of the gene was found in cancer cell lines from these tissue origins (11). It will be also important to determine how virally encoded IL-10-like cytokines participate in cell infection and transformation, and through which receptor(s) these viral cytokines function. It should be pointed out that the human IL-24 gene (mda-7), when ectopically expressed, was reported to have growth inhibitory effect on some cancer cell lines (29), which is in paradox with our finding that the gene is both induced by the ras oncogene and overexpressed in colon cancer tissues (data not shown). With the finding that IL-24 is a secreted cytokine, as we predicted previously, and it functions through its specific heterodimeric receptors, the apparent contradiction may now be clarified by more mechanistic experimentation.

Taken together, our work presented here should help to shed light on future work on how aberrations in the expression of IL-24 or other members of IL-10 family of cytokines and their receptors could contribute to neoplastic cell transformation.

Acknowledgments—We are grateful to Dr. Lynn Matrisian and Emily Thomas for critical reading of the manuscript and to the Molecular Recognition Core facility of Vanderbilt-Ingram Cancer Center for generating the monoclonal antibody to IL-24. In concordance with the nomenclature of established members of the IL-10 family of cytokines, MDA-7/MB0-5 has been renamed as interleukin 24 (IL-24) with the approval of the HUGO Gene Nomenclature Committee.

REFERENCES


FIG. 7. Inability of IL-24 to inhibit LPS-induced TNF-α production from PBMCs. The freshly prepared human PBMCs were either mock treated or treated with 80 ng/ml LPS for 4 h in the absence and presence of IL-10 (20 ng/ml), IL-24-His (100 ng/ml), or both cytokines as indicated. TNF-α secreted into the media was quantified with ELISA. Unlike IL-10, IL-24-His had little inhibitory effect on LPS-induced TNF-α production by PBMCs. The error bar represented the standard deviation of duplicate data obtained for each data point within the same experiment. The result shown are representative of at least four independent experiments.

TABLE I

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