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IL-24 Transgenic Mice: In Vivo Evidence of Overlapping Functions for IL-20, IL-22, and IL-24 in the Epidermis

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IL-20 and IL-24 share two different heterodimeric receptors consisting of either IL-20R1 or IL-22R1 and a common IL-20R2 subunit, whereas IL-22 signals through IL-22R1/IL-10R2. However, until now, only IL-20 and IL-22 have been proven to play important roles in vivo in the epidermis where all four receptor subunits are expressed. In this study, we show that IL-24 transgenic mice manifest many similar phenotypes to that of IL-20 and IL-22, including neonatal lethality, epidermal hyperplasia, and abnormality in keratinocyte differentiation. These results support a largely redundant role in epidermal functions for IL-20, IL-22, and IL-24, which seem to be IL-22R1 dependent. Moreover, we show that IL-24 transgenic mice exhibit infiltrating macrophages in the dermis with concomitant increases in MCP-1 production from both keratinocytes in the epidermis and immune infiltrates in the adjacent dermal layer below. Furthermore, we demonstrate that the homodimeric IL-20R2 soluble receptor is a potent blocker for IL-24 and can be used to further dissect the crosstalk among the IL-20 family of cytokines in normal development as well as in autoimmune diseases. *The Journal of Immunology*, 2010, 184: 1793–1798.

It is well established that the newly discovered IL-20 family of cytokines all signal through heterodimeric cell surface receptors consisting of four types of receptor subunits, IL-20R1, IL-22R1, IL-10R2, and IL-20R2 (1, 2). Unlike heterodimeric receptors with the IL-10R2 subunit, heterodimeric receptors consisting of IL-20R2 are quite promiscuous, with IL-19, IL-20, and IL-24 all able to bind to and signal through IL-20R1/IL-20R2 and IL-20 and IL-24 also able to signal through IL-22R1/IL-20R2 (3, 4). Thus, the significant receptor sharing among these cytokines based on in vitro biochemical assays with cultured cells has raised questions about whether the three cytokines have redundant biological functions in vivo or whether they may use the same receptors for different biological end points in a tissue-specific or temporally regulated manner.

Supported by the evidence in receptor expression patterns, keratinocytes have been identified as a major target cell type for the IL-20 family of cytokines (4–7). In vitro assay using reconstituted keratinocytes have been identified as a major target cell type for the IL-20 family of cytokines. However, convincing functional evidence in vivo from transgenic (Tg) mouse model so far has only linked IL-20 and IL-22 directly in epidermal proliferation and differentiation (5, 6), whereas IL-19 Tg mice purportedly did not manifest any overt skin phenotype (11). In contrast, structural basis for receptor sharing among IL-19, IL-20, and IL-24 has not been investigated in detail. Overall, amino acid sequence homology among these three cytokines is only ~20–35% (2, 3). Although IL-24 was also shown to be able to bind to IL-20R2 alone expressed on the cell surface without the ability to signal (4), both IL-19 and IL-24, but not IL-20, reportedly could bind to a dimeric soluble IL-20R2-Fc (sIL-20R2-Fc) fusion protein with low affinity compared with their native heterodimeric receptors (11).

In this study, we show that IL-24 Tg mice targeted to the skin exhibit many phenotypes similar to that of IL-20 and IL-22 with profound effects on epidermal proliferation and differentiation. We show that in IL-24 Tg mice, infiltrating macrophages were found to congregate in the dermal layer right beneath the epidermis, which correlated with MCP-1 induction from both keratinocytes in the epidermis and infiltrating leukocytes in the dermal layer. Furthermore, we demonstrate that sIL-20R2-Fc fusion protein can bind to IL-24, but not to IL-19 and IL-20, with high affinity and is a potent IL-24 antagonist.

Materials and Methods

Cell lines

HEK 293T cell lines expressing AP (alkaline phosphatase) fusion proteins, Cos E5 cells (GenHunter, Nashville, TN), and baby hamster kidney (BHK) 21 (IL-20R1/IL-20R2) cell line were cultured as described previously (4). Chinese hamster ovary (CHO) cells expressing sIL-20R2-Fc was cultured in IMDM (Invitrogen, San Diego, CA), containing 10% FBS (Atlanta Biologicals, Norcross, GA) and 1% penicillin-streptomycin. Serum-free production of sIL20R2-Fc was carried out in shake flask by culturing the cell in SFM4CHO medium (Hyclone, Logan, UT).

Generation of IL-24 Tg mice

For creating IL-24 Tg mice targeted to the skin, full-length FISP cDNA clone (Genbank accession no. BB867056) was purchased from Riken’s Institute and Centers, Tokyo, Japan. The complete coding region of mouse IL-22 (12) was PCR amplified and subcloned into the Nhel site of pK5 mammalian expression vector under bovine keratin 5 promoter control.
The construct was verified by sequencing before microinjection. Generation of IL-24 Tg mice was carried by the Vanderbilt Transgenic Cell Shared resource. Briefly, female B6D2 mice were obtained from Harlan (Indianapolis, IN) at 3–5 wk of age. These females were superovulated and mated with B6D2 males for embryo production. The embryos that survived injection were transferred into the oviduct of CD-1 pseudo-plugged females (Charles River Laboratories, Wilmington, MA).

**Genotyping for IL-24 Tg mice**

A total of 0.5 cm of pup tails were digested with proteinase K (20 μg/ml) and incubated overnight at 50°C–55°C with gentle shaking, followed by iso-propanol precipitation. The genomic DNA was then washed with 70% ethanol and dissolved in 100 mM Tris-HCl, pH 8.0. The 450-bp fragment specific to FISP transgene was detected by PCR using primers, t-flap: 5′-GCTAAGTCTCCCACCCAGAAGATC-3′ that anneals to the 5′ end of FISP and r-ext: 5′-GAACTGACCTCAAGACCTTGAATTTG-3′ that anneals to 3′ end coding site of bovine keratin 5 expression vector. The 130-bp control genomic DNA locus Bid was amplified by primers, 17B14: 5′-CCGAAATGTCCCATAAGAG-3′ located in murine Bid intron 3 near E3/I3 boundary and 17B12: 5′-GAGATGGACCAACACATC-3′ located in exon 3.

**Histological analysis and immunohistochemistry**

Skin samples from the backs of IL-24 Tg pups (neonatal lethal) and normal control littersmates (1 d old) were fixed in 10% neutral buffered formalin, paraffin-embedded, and sectioned at 5 μm and stained with H&E. For immunohistochemistry, the sections were rehydrated and placed in heated Target Retrieval Solution (DakoCytomation, Carpinteria, CA) for 20 min. Endogenous peroxidase activity was quenched with 0.3% hydrogen peroxide and a protein block treatment (DakoCytomation) was performed prior to primary Ab addition. Tissues were incubated with rabbit anti-IL-24 Ab (1:50 dilution, anti-keratin 5 Ab (Covance Research Products, Princeton, NJ) at 1:2000 dilution, anti-keratin 5 Ab (Covance Research Products) at 1:500 dilution, or anti-keratin 6 Ab (Covance Research Products) at 1:500 dilution, or anti–Ki-67 Ab (Vector Laboratories, Burlingame, CA) at 1:2000 dilution, for 60 min, or rat anti-mouse F4/80 (Serotec, Raleigh, NC) at 1:30 dilution for overnight. Goat anti-mouse CD3 (catalog SC-1127; Santa Cruz Biotechnology, Santa Cruz, CA) and goat anti-mouse MCP-1 (catalog AB-479-NA; R&D Systems, Minneapolis, MN) were both used at 1:500 dilutions. The rabbit envision +DAB+ system (DakoCytomation) and Dako Envision+ HRP/DAB System (DakoCytomation) were used to produce localized and visible staining with corresponding secondary Abs. Slides were lightly counterstained with Mayer’s hematoxylin, dehydrated, and mounted with coverslip.

**Alkaline phosphatase and IL-24–AP receptor binding and cell staining assays**

Quantitative and in situ receptor binding studies on mouse tissues, BHK cells stably expressing IL-20R1/IL-20R2, and Cos E5 cells transiently transfected with IL-20R1/IL-20R2 expression vectors were carried out using AP assay reagent A and AP assay reagent S (GenHunter), respectively, as previously described (4).

**RESULTS**

**IL-24 induces abnormality in epidermal differentiation and proliferation**

To understand the biological functions of IL-24 in vivo, we generated IL-24 Tg mice using mouse IL-24 cDNA under bovine keratin 5 promoter control (Fig. 1A). As expected, IL-24 Tg mice resembled many phenotypes of IL-20, including neonatal lethality within hours of birth, smaller body size, and shiny and dark appearance with wrinkled skin (Fig. 1B). Histological analysis of the skin revealed compact stratum corneum and marked hyperplasia in the epidermis of Tg mice compared with wild-type (WT) littermates (Fig. 1C), again similar to IL-20 and IL-22 Tg mice. On average, the epidermis...
of IL-24 Tg mice was twice as thick as the epidermis of WT controls (Fig. 1D). Immunostaining with anti-murine IL-24 Ab confirmed diffusive pattern of IL-24 protein expression throughout the skin (both epidermal and dermal layers) of the Tg mice, consistent with the nature of IL-24 being a secreted cytokine (Fig. 1C).

IL-24–AP ligand-affinity staining showed that IL-24 receptor expression pattern was unaltered in the epidermis of IL-24 Tg mice (Fig. 2A), despite marked epidermal hyperplasia. Furthermore, the epidermis of IL-24 Tg mice retained keratin 5 expression in both suprabasal and basal layers in addition to hair follicles, as in the normal controls (Fig. 2B). To provide further evidence for IL-24–induced keratinocyte proliferation, keratin 6, which is often associated with keratinocyte proliferation (e.g., during wound healing), and Ki-67, which is a marker for proliferating cells, were also analyzed by immunohistochemistry. Compared with WT controls, IL-24 Tg skins exhibited strong positive staining for keratin 6 throughout suprabasal and basal layers, much like that in IL-20 Tg mice (5), whereas Ki-67 staining was restricted mostly in the basal layer (Fig. 2B). These results suggest that, like IL-20 and IL-22, IL-24 functions either directly or indirectly as a potent mitogen for keratinocytes in vivo and the abnormal keratinocyte proliferation appears originate from the basal layer of the epidermis.

**IL-24 induces localized chemokine production and macrophage infiltration in the dermis**

In addition to the dramatic effects of IL-24 on keratinocytes in the epidermis, some of which resemble phenotypes observed in psoriatic skins, we also examined whether there were any immune infiltrates in the Tg skins. Immunohistological analysis was performed with Abs against CD3 and F4/80 to detect any differences in infiltrating T cells and macrophages, respectively. Although little difference in CD3-positive T cell counts was visible, a significant number of F4/80 positive cells were detected in the dermis of IL-24 Tg pups (Fig. 3), similar to the finding with IL-22 Tg mice (6). Apart from nearly all the phenotypes in the epidermis examined, infiltrating macrophages appear to be the major difference observed in the skin between IL-24 and IL-20 Tg mice where no immune infiltrates were detected (5). Thus, it appeared that IL-24 either directly or indirectly induced certain chemokines, which elicited the migration of monocytes to the dermal layer right beneath proliferating keratinocytes. To this end, we first focused on MCP-1, a major monocyte chemotactic that has been linked to psoriasis (15). Immunohistochemical staining with goat anti-mouse MCP-1 revealed marked induction of MCP-1 in IL-24 Tg skin tissues in comparison with that of normal controls (Fig. 3). Of great interest was the finding that, in addition to many immune infiltrates that stained positive for MCP-1, keratinocytes in the entire epidermis of IL-24 Tg mice were the source of MCP-1 secretion (Fig. 3)

**sIL20R2-Fc is potent blocker of IL-24**

Although Tg mouse work starts to shed light on the complicated scheme of the receptor crosstalk network among the IL-20 family of cytokines, further dissection of these cytokine actions requires the availability of antagonists specific to individual cytokines. To help further understand the degree of redundancy in biological functions and promiscuity in receptor use among the IL-20 family of cytokines, we sought to develop an IL-24–specific antagonist.
Based on our earlier finding that IL-24 could bind to IL-20R2 directly (4), we envisioned that a homodimeric sIL-20R2 might be a potent blocker of IL-24 signaling. To this end, we fused the extracellular domain of human IL-20R2 to human IgG1 Fc. The resulting sIL-20R2-Fc fusion protein was expressed in CHO cells and purified from the conditioned medium to homogeneity via protein A affinity column. Both immunoprecipitation-Western blot and kinetic binding studies to its cell surface receptor IL-20R1/IL-20R2 showed that sIL-20R2-Fc indeed is a potent binder and blocker of IL-24 with an inhibition constant of 11 nM (Fig. 4), which is similar to the $K_d$ of IL-24 to its native heterodimeric receptors (4). Interestingly, sIL-20R2-Fc had little effect on IL-19 and IL-20 bindings to IL-20R1/IL20R2, even when used at a concentration 10 times of IL-24 $K_d$, where IL-24 receptor binding becomes nearly completely blocked (Fig. 5).

**Discussion**

The essentially identical phenotypes in Tg mouse models for IL-20, IL-22, and IL-24 suggest that the three related cytokines play a redundant role in keratinocyte differentiation and proliferation. The significantly increased thickness of the epidermis with concomitant reduction in the stratum corneum layer seen in all three Tg mice is suggestive of either hyperproliferative keratinocytes or delayed keratinocyte terminal differentiation, or both. Importantly, characterization of IL-24 Tg mice also revealed significant macrophage infiltrates underneath the hyperproliferating epidermis that is characterized by the expression of keratin 6 and Ki-67. Although a study from IL-20 Tg mice failed to show any dermal infiltrates (5), immune infiltrates were noticed in IL-22 Tg mice (6). In this study, we were able to trace the potential cause for macrophage migration to localized MCP-1 production from both keratinocytes in the epidermis and immune infiltrates in the dermal layer below. Indeed, keratinocytes have been shown to be the major source of MCP-1 production in human psoriasis that is restricted to the dermal layer (15). Although our finding suggests that IL-24 can either directly or indirectly induce chemokine production in the skin of the Tg mice, it does not necessarily prove that MCP-1 is involved in the skin phenotype observed, because IL-20 Tg mice seemed to lack any immune infiltrates in the dermis (5). Furthermore, in human psoriasis, IL-24 appears to be produced by infiltrating monocytes/macrophages (9), so chemokines may have to act upstream of IL-24 in the first place, in contrast to the findings with the Tg mouse models. It is also interesting to note that, as in previous studies that showed little chemokine induction by both IL-20 and IL-22 in vitro (6, 16, 17), no MCP-1 induction by IL-24 could be detected by Western blot with cultured human keratinocyte cell line, HaCat (data not shown). These observations underscore the importance of studying the complicated cytokine networks that are involved in the skin inflammation, such as psoriasis, in the context of different

**FIGURE 3.** Analysis of immune infiltrates and chemokine production in IL-24 Tg skins. Paraffin-embedded skin sections (original magnification ×40) from both IL-24 Tg pups and WT control littermates were analyzed with CD3, F4/80, and MCP-1–specific Abs to visualize the presence of T cells, infiltrating monocytes (macrophages), and chemokine secretion, respectively. Note the presence of macrophages (indicated by arrowheads) in the dermal region of IL-24 Tg skins, which correlated well with marked induction of MCP-1 (brown color) from both keratinocytes in the epidermis and immune infiltrates in the dermal layer below (best viewed at >5× magnification of the figure). The results were representative of at least three independent experimental repeats.

**FIGURE 4.** sIL-20R2-Fc binds to IL-24 with high affinity and is a potent IL-24 antagonist. A, Immunoprecipitation-Western blot analysis of sIL-20R2-Fc binding to IL-24–AP. AP alone was used as a negative control. Either IL-24–AP or AP conditioned medium (both 1 U/ml) was incubated with equal amount of purified sIL-20R2-Fc. The IL-24–AP/sIL-20R2-Fc complex was pulled down by either protein A beads or mAb against AP linked to Sepharose, and detected by Western blots with Abs against either IL-24 or IgG-Fc, respectively, as indicated. Note only IL-24–AP, but not AP alone, formed complex with sIL-20R2-Fc, which could be reciprocally pulled down by either protein A beads that bind to Fc domain or AP Ab beads that bind to AP moiety. B, Kinetic study of sIL-20R2-Fc inhibition of IL-24 binding to its cell surface receptor IL-20R1/IL20R2 expressed in BHK cells. Each data point was from average of triplicate measurements. The data were analyzed with kinetic program “HYPER,” which gave an inhibition constant of 11 nM for sIL-20R2-Fc.
interacting cell types found in skin. Genetic screens in humans for single nucleotide polymorphisms at IL-24, IL-20R1, and IL-20R2 loci showed little evidence that these genes are linked to increased susceptibility to psoriasis (18, 19). Therefore, one must be cautious in extending the generalization of these cytokine actions to human diseases beyond the phenotypes seen with the Tg mice.

It is interesting to note that IL-19 Tg mice purportedly failed to manifest any overt skin phenotype (11). These results would support the fact that the epidermal hyperplasia caused by both IL-20 and IL-24 is likely mediated through the IL-22R1/IL-20R2 receptor through which IL-19 fails to signal. Consistent with this prediction was the finding that IL-22 Tg mice also exhibited similar phenotypes to those of IL-20 and IL-24 (6). Because IL-22 signals through IL-22R1/IL-10R2, the combined data from these Tg mice seemed to suggest that the IL-22R1 may be essential for mediating keratinocyte functions, which could be distinct from yet to be identified biological functions mediated through IL-20R1/IL-20R2. This revelation may have major implications for therapeutic strategies for treating autoimmune skin diseases. For example, therapeutic Abs to IL-22R1 may be superior to Abs to IL-20R2 or heterodimeric soluble receptor-Ig fusion proteins because targeting IL-22R1 may simultaneously block IL-20, IL-22, and IL-24 without affecting IL-19 or signaling through IL-20R1/IL-20R2. Recent availability of IL-20R2 knockout mice (20), in which the functions of IL-19, IL-20, and IL-24 should all be abolished, will be useful to confirm or rule out the importance of these cytokines in epidermal functions. Likewise, IL-22R1 and IL-20R1 knockout mice, once available, will help further pinpoint or clarify the biological functions of the IL-20 family of cytokines in vivo.

The results from IL-24 Tg mice further confirmed that IL-24 is a classic cytokine like IL-20 and IL-22. Although it is possible that IL-24 may induce growth arrest or apoptosis for certain types of cells in vitro (21, 22), the postnatal lethality and psoriatic skin phenotype as a result of hyperplasia in the epidermis observed for IL-24 Tg mice raise potential serious safety issues for using IL-24 (also known as MDA-7 before it was shown as a cytokine) as an anticancer agent in gene therapy (1, 23).

Our finding that the dimeric sIL-20R2-Fc fusion protein is a potent IL-24–specific antagonist that is largely consistent with a previous estimate via a nonquantitative and indirect receptor binding assay (luciferase reporter assay), in which sIL-20R2-Fc was purported (without actual data support) to inhibit both IL-24 and IL-19, albeit much poorly compared with the heterodimeric soluble receptors (11). However, unlike heterodimeric soluble receptor-Ig fusions, which could not differentiate IL-24 from IL-20 (11), our results indicated that sIL-20R2-Fc not only can be more easily produced, but also is a potent IL-24–specific antagonist. Although Tg mouse studies support an overlapping function of IL-20 and IL-24 in normal epidermal differentiation, both the mouse model for IL-23–mediated psoriasis (10) and studies on human psoriasis tissue samples (9) seemed to suggest that the two cytokines might play different roles in the development of autoimmune diseases. Thus, the availability of sIL-20R2-Fc and its high specificity against IL-24 offers not only another research tool for a more in-depth understanding of the IL-20 family of cytokines, but also a potentially therapeutically important biologic agent in tackling autoimmune diseases.

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Disclosures
The authors have no financial conflicts of interest.
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