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Myeloid cells Migrate in Response to IL-24

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Abstract

IL-24 (melanoma differentiation associated gene 7 product) is a member of the IL-10 cytokine family that has been reported to possess anti-tumor activity. IL-24 is produced by immune tissues and its expression can be induced in human peripheral blood mononuclear cells by pathogen-associated molecules. While immune cells are known to produce IL-24, the response of immune cells to IL-24 is unclear. Using recombinant human IL-24, we demonstrated that IL-24 induces human monocyte and neutrophil migration, in vitro. An in vivo chemotaxis model showed that IL-24 attracted CD11b positive myeloid cells. To further characterize the chemotactic IL-24 response and type(s) of receptor(s) utilized by IL-24, we treated monocytes with signaling pathway inhibitors. IL-24-induced migration was reduced by pertussis toxin treatment, thus implicating G-protein coupled receptors in this process. Additionally, MEK and JAK inhibitors markedly decreased monocyte migration toward IL-24. These results suggest that IL-24 activates several signaling cascades in immune cells eliciting migration of myeloid cells, which may contribute to the known anti-cancer effects of IL-24.

Keywords

IL-24; Mda-7; monocyte; neutrophil; migration

1. Introduction

IL-24 (or melanoma differentiation associated gene 7 product, mda-7) is best known as a tumor suppressor. It is a member of the IL-10 cytokine family, other family members include IL-20, IL-22, IL-26, IL-28 and IL-29. Further it is produced by immune cells and keratinocytes [1, 2]. Expression of IL-24 can be induced by stimulating peripheral blood mononuclear cells (PBMC) with phytohemagglutinin (PHA), lipopolysaccharide (LPS), IL-4 or influenza A virus infection [3, 4]. IL-24 induces the expression of proinflammatory cytokines such as IL-6, IFN- γ and TNF- α suggesting a Th1 bias despite its amino acid sequence homology to the classic Th2 cytokine, IL-10 [5].

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Two heterodimeric receptors have been identified for IL-24 on epithelial and stromal cells [6, 7]: IL-20R2/IL-20R1 and IL-20R2/IL-22R. IL-24 binds to IL-20R2 alone, but heterodimerization with IL-20R1 or IL-22R increases the binding affinity and is necessary for receptor mediated signal transduction. IL-20R1, IL-20R2 and IL-22R1 are not expressed by immune cells[7]. Nevertheless, treating peripheral blood mononuclear cells with IL-24 induces those cells to produce many pro-inflammatory cytokines, suggesting that IL-24 interacts with as yet unidentified receptors on PBMC [5].

Adenovirus delivery of IL-24 (Ad-24) to the intracellular compartment, which circumvents receptor utilisation, induces selective apoptosis in cancer cells, but not normal somatic cells; the exact mechanisms of selective tumor cell death are not fully understood [8–12]. Ad-24 administration to most tumors, including solid tumor e.g., pancreatic, prostate, melanoma, non-small cell lung carcinoma and [13] leukemia cell lines, leads to cell-specific apoptosis. Ad-24 results in the activation of a number of cellular signaling molecules including the caspase cascade, PKR, p38, STAT3, PI3K, GSK-3, ILK-1, BAX, BAK, Fas, DR4, TRAIL, iNOS, IRF-1 and IRF-2 [13–16] and can result in arrest at the G2/M cell cycle phase [17]. Importantly, these signaling molecules are not uniformly expressed by neoplastic and normal cells, suggesting that the tumor selective apoptotic activity of IL-24 is due to the level of signal not presence of signal [18].

IL-10 and IL-20 have been demonstrated to induce cell migration by both direct and indirect methods [19–23]. IL-10 has been shown to enhance Langerhans cell migration from skin, however the molecular mechanism for this effect was not demonstrated [19]. Several indirect mechanisms for IL-10 induced cell migration have been reported, including IL-10-induced increase in CCL2 (MCP-1) expression by human monocytes [23], CCR6 expression by Langerhans cells [21] and cytoskeletal reorganization subsequent to CXCL12 (SDF-1) treatment of B-cells[22]. IL-20 signals through IL-20R1 and IL-20R2 on endothelial cells to induce phosphorylation of MAP kinase family members, which correlated with HUVEC migration and vascular tube formation [20]. IL-24, by stimulating IL-22R on endothelial cells, has been shown to inhibit endothelial cell differentiation and migration [24]. Further, IL-24 (at ng/ml concentrations) has been shown to inhibit TGF α -induced keratinocyte proliferation and migration through the IL-20R1/IL-20R2 complex [25]. The effect of IL-24 on immune cell migration has not been reported.

In this study, we investigated the ability of IL-24 to induce leukocyte migration both in vitro and in vivo. We further evaluated the signaling cascade(s) initiated by IL-24 stimulation of monocytes in an effort to elucidate the class of receptor(s) utilized by IL-24 on PBMCs.

2. Materials and Methods

Unless otherwise indicated supplies were obtained from Sigma-Aldrich St. Louis, MO. Recombinant human IL-24 (R&D systems), recombinant human CCL2 (Peprotech, Rocky Hill, NJ).

2.1 Human monocytes and neutrophils

Primary human leukocytes were isolated from fresh normal donor leukapheresis packs under an approved human subjects protocol as previously reported [26]. The blood was centrifuged (600 x gravity for 30 minutes) through Ficoll-Hypaque (Sigma), and peripheral blood mononuclear cells (PBMCs) collected at the interface were washed with PBS and centrifuged through a one step 23% isoosmotic Percoll (Pharmacia, Uppsala, Sweden) gradient (900 x gravity for 30 minutes). The enriched monocyte population was recovered from top of the percoll cushion. The monocyte population is >85% pure based on non-specific esterase stain. The pellet from the Ficoll-Hypaque separation is mixed with 3%

dextran-500 (Pharmacia) and allowed to settle for 30 minutes, the contaminating red blood cells settle leaving 95% granulocytes at the top of the dextran cushion. Following isolation, neutrophils were used for in vitro chemotaxis assays immediately while monocytes were stored overnight at 4°C in DMEM containing 10% FCS.

2.2 Migrations assays

In vitro chemotaxis assays were performed as described previously [26]. Micro-Boyden chambers were supplied by Neuro Probe (Gaithersburg, MD). Briefly, cells were resuspended at 1 million/ml in chemotaxis media (RPMI containing 1% bovine serum albumin (Sigma) and 25 mM HEPES pH 8 (Gibco)). Standard polycarbonate track-etch (PCTE) membranes (5 μ m size) were used to separate resuspended cells in the upper wells from chemoattractants diluted chemotaxis media. Neutrophils were incubated for 60 minutes at 37°C in the micro-Boyden chambers while monocytes were incubated for 90 minutes. After the cell specific migration time, the membrane was removed from the micro-Boyden chamber, the cell side wiped to remove the cells that did not migrate through the membrane and the membrane with migrated cells was H&E stained. The number of cells migrating was determined by microscopy of the H&E stained membrane at 400 X power. Chemotactic Index was calculated by dividing number of cells counted in the induced migration by number of cells counted in the spontaneous migration. A minimum of 6 determinations were made for each condition. Mean values and standard errors of the mean (SEM) are reported. Graphics were produced and statistical analysis performed using GraphPad Prism. For the “checkerboard” chemokinesis assay, monocytes were pretreated at 37°C with recombinant IL-24 at the indicated concentrations for 30 minutes before being added to the upper wells of the micro-Boyden chamber. To inhibit the signaling of GiCPR we used 100 ng/ml pertussis toxin (List Biological Laboratories, Campbell, CA). 50 μ M AG 490 (Calbiochem, La Jolla, CA) was used as a JAK family inhibitor and 50 μ M U0126 (Sigma) as a MEK1 and MEK2 inhibitor. Cells suspended in medium were treated with inhibitors at the specific concentrations for 30 min at 37°C in a water bath.

2.3 In vivo chemotaxis assay - Air Pouch

C57BL/6 mice were provided by Animal Production Area of the NCI (Frederick, MD). NCI-Frederick is accredited by AAALAC International and follows the Public Health Service Policy for the Care and Use of Laboratory Animals. Animal care was provided in accordance with the procedures outlined in the “Guide for Care and Use of Laboratory Animals” (National Research Council; 1996; National Academy Press; Washington, D.C.) The method was adapted from Sironi et al [27, 28]; 3 ml of air was injected subcutaneously into the dorsal surface of the test mouse on day one to form an air pouch. On day three, 2.5 mls of sterile air were be injected into the same site to re-enforce the air pouch. On day six, 1 ml of sterile eliciting agent diluted in HBSS w/o Ca^{2+} and Mg^{2+} was injected into the air pouch. The eliciting agent concentrations were either HBSS or 1000 ng/ml CCL2, 10, 1.0, 0.1 ng/ml IL-24. After 24 hours the test animals were be euthanized by CO_2 and the elicited leukocytes collected for further analysis. Each treatment group contained 5 mice. 5 experiments were conducted with HBSS and 1000 ng/ml CCL2. 4 experiments were conducted with either 10, 1.0 or 0.1 ng/ml IL-24. Mean values and standard errors of the mean (SEM) are reported as are two-tailed Student t-test values comparing test samples to HBSS control samples. Graphics were produced and statistical analysis performed using GraphPad Prism.

2.4 FACS analysis

The cells were fixed by 1% w/v paraformaldehyde in PBS and stained with 0.5 μ g/ml antibodies against CD3 ϵ (B&D, cat# 553062), CD11b (B&D, cat # 557397), CD45 (BioScience, cat# 48-0451-82), Ly6C (Biolegend, cat#128006), Ly6g (Biolegend, cat#

127614) and F4/80 (eBioscience, cat # 48-4801). Cells were handled in FACS buffer (DPBS without calcium and magnesium, 1% Fetal calf serum, 1% goat sera, 0.02% sodium azide). FACS analysis was performed on Canto II (BD Biosciences) using Diva software. Analysis was performed using FlowJO (Tree Star, Inc.). Mean values and standard errors of the mean (SEM) are reported as are two-tailed Student t-test values comparing test samples to HBSS control samples. The number of repeats was at least 4 for each eliciting agent.

3. Results

3.1 IL-24 induces human monocyte and neutrophil migration in vitro

Using a recombinant human IL-24, we set up in vitro cell migration assays. By using log dilutions of cytokine; we observed maximal migration in the range of 0.125–0.0125 ng/ml. (Fig. 1). One mechanism to demonstrate the difference between directed migration or chemotaxis and increased random movement or chemokinesis is to add IL-24 to the cells before performing a migration assay. If the migration is directional then when cells are exposed to concentrations at or below the migratory maximum concentration (0.125 ng/ml), the cells should migrate without significant changes from the media control. Also, if the migration is directional then when the cells are mixed with IL-24 concentrations higher than the migratory maximum concentration the cells should not migrate toward lower concentrations of IL-24. Shown in Table I, monocyte migration induced by IL-24 is not exclusively directional but contains both chemotactic and chemokinetic migration. This is indicated by the increase in migration observed when the cells are pretreated with 0.001 ng/ml IL-24 followed by exposure to 0.12 ng/ml of IL-24 and the fact that pretreatment with 1.2 ng/ml did not completely block migration although spontaneous migration was reduced. Our results demonstrate the ability of IL-24 to induce human monocytes and neutrophils migration *in vitro*.

3.2 IL-24 induces leukocyte migration in vivo

We next evaluated whether IL-24 recruited cells *in vivo*. Based on earlier studies showing a need for 10 fold more chemoattractant *in vivo* than that needed for maximal *in vitro* activity [28], we evaluated a range of IL-24 from 0.1 ng to 10 ng per mouse. As shown in figure 2, The 0.1 ng dose of IL-24 induced a 2-fold increase in total cells recovered while a 1000 ng dose of CCL2 induced a 1.5-fold increase, both increases are statistically significant. These results indicate that IL-24 can induce cell migration *in vivo* and *in vitro*.

3.3 IL-24 induces subsets of Leukocytes to migrate

In an effort to evaluate the subsets of leukocytes recruited by IL-24 into the mouse air pouch, we performed FACS analysis. Proportionally, there fewer CD3 positive CD45 positive leukocytes recruited by IL-24 than by CCL2, see figures 3A and B. While the majority of the cells recruited by either CCL2 or IL-24 are CD11b positive myeloid cells, IL-24 recruits statistically more CD11b positive F4/80 positive resident macrophage, see figures 3C and D. Both IL-24 and CCL2 recruited similar proportions of CD11b positive, Ly6G high, neutrophils (data not shown).

3.4 Classes of IL-24 migratory receptors on leukocytes

To categorize the classes of receptors used by IL-24 on leukocytes, we treated monocytes with signal cascade inhibitors. Because the majority of chemoattractant receptors are G protein-linked receptors that are sensitive to inhibition by pertussis toxin, we investigated the effect of pertussis toxin on IL-24-induced migration. As shown in Fig. 4A the migration induced by IL-24 was sensitive to pertussis toxin treatment, thus, a G protein-linked receptor of the G α subfamily is implicated in IL-24-induced cell migration.

Many growth factor receptors that have secondary migratory effects are known to activate members of the MAPK family [29]. The receptors for IL-24 expressed by endothelial cells, IL-20R2/IL-20R1 and IL-20R2/IL-22R, are known to signal through the JAK/STAT pathway activation [8, 11, 12, 30]. We therefore tested inhibitors of these pathways and found that MEK, hatched bars compared to solid bars, (Fig. 3B) and JAK, hatched bars compared to solid bars, (Fig. 3C) inhibitors significantly decrease monocyte migration induced by IL-24.

4.0 Discussion

IL-24 induces human monocyte and neutrophil migration *in vitro* and leukocyte migration *in vivo*

We have observed that IL-24 attracts monocytes and neutrophils at very low (0.0125ng/ml) concentration *in vitro*. This indicates that IL-24 is a potent *in vitro* chemoattractant of myeloid cells and that the effect of IL-24 is likely to be due to its direct action on myeloid cells since the *in vitro* assay, at most, requires 90 minutes of exposure to the IL-24 to be complete. Using the murine air pouch migration model, we observed that injection of recombinant IL-24 increased leukocyte infiltration 2-fold *in vivo*. FACS analyses of attracted cells showed that the majority of the cells recruited into a mouse air pouch are CD45 positive and CD11b positive myeloid cells ($\approx 80\%$) regardless of the eliciting agent, however, double the total cell number of cells was recruited by IL-24 vs. buffered saline indicating that IL-24 recruited myeloid cells *in vivo*. IL-24 attracts 10% more CD11b positive, F4/80 positive cells than either CCL2 or buffered saline. Expression of F4/80 is reported to identify a more mature, connective tissue, resident macrophage [31], like the macrophage found in the skin. Others have shown that over expressing IL-24 in mouse skin increased CCL2 expression and macrophage infiltration into the dermis [32]. Our data is in agreement with He and Liang's observation that over expressing IL-24 in mouse skin did not increase lymphocyte recruitment [32]. Taken together, these observations suggest that IL-24 both directly and indirectly contributes to the recruitment of macrophages.

Both CCL2 and IL-24 recruit equal numbers of CD11b positive Ly6G high granulocytes. This observation in conjunction with an earlier observation that IL-24 stimulates granulocytes to produce IFN- γ , IL-12 and nitric oxide, which leads to an innate response restricting salmonella infection and contributes to activation of CD8 responses [33], suggests that IL-24 might both recruit and activate neutrophils. Further, our data permits the speculation that localized production or administration of IL-24 could drive myeloid cell invasion of tumor sites. Together these findings lead us to propose an additional basis for the anti-tumor effect of IL-24, namely recruitment of neutrophils and macrophage by IL-24 not only promotes a Th1 response, but may also promote cytotoxic T-cell activation [5, 33].

Classes of IL-24 migratory receptors on leukocytes

In this study we show that the oncolytic cytokine, IL-24, can induce monocyte migration at low concentrations (pg/ml) and this migration is sensitive to inhibition by Gialpha-protein, JAK and MEK inhibitors. No single chain receptor utilizes all these pathways, suggesting that multiple IL-24 receptor types are expressed on myeloid cells or that the IL-24 receptor on myeloid cells is a multi-component receptor. Our observation that an inhibitor of JAK kinases blocks monocyte movement toward IL-24 suggests that an IL-10R family member could be involved in cell migration [34] since IL-10R1 and R2, IL-20R1 and R2, IL-22R1, and IFN- λ R1 each utilize different JAK kinases to transmit signals to the STATs. IL-10R2 is a common low affinity ligand binding and signaling chain in IL-10, IL-22, IL-26, IL-28A (IFN- λ 2), IL28B (IFN- λ 3) and IL-29 (IFN- λ 1) cell activation and it is expressed by most cell types [35]. This is in contrast to other members of the IL-10R family, which have more

restricted expression profiles. It is appealing to hypothesize that IL-10R2 is also a component of the IL-24 signaling receptor on leukocytes, but as it is unlikely to be the high affinity receptor we are unable to confirm this. Earlier studies to characterize the viral IL-10 receptor complex illustrated the need for at least two components of the receptor in order to obtain both binding and signal transduction [36]. At this time we do not have a candidate for the high affinity IL-24 receptor on myeloid cells. Further, our data showed that inhibiting MEK, which is common to both the G-protein and IL10R signal cascade, also inhibited the IL-24 induced migration of monocytes. Taken together, our data suggests that the IL-24 receptor complex on myeloid cells is a multi-component receptor using G-protein, JAK and MEK signaling pathways. This is distinct from the characterized IL-24 receptor complexes expressed by endothelial cells, which are composed of IL-20R1/R2 or IL-22R1/IL-20R2 and do not depend on G-protein signal transduction. The fact that IL-24 appears to have a leukocyte specific receptor complex is not unique among the IL-10 family of cytokines. IL-19, and IL-20, [32, 34] use the same promiscuous receptor complex as IL-24 (IL-20R1/R2) on keratinocytes, but leukocytes don't express those receptors. However, IL-19 induces monocytes to express TNF and IL-6 [37] and IL-20 induces neutrophil migration [38], suggesting that a leukocyte specific receptor complex may exist for a subset of the IL-10 family of cytokines. We therefore hypothesize the existence of a novel receptor complex consisting of IL10R2 plus a novel high affinity binding receptor for IL-24, IL19 and IL-20 on leukocytes, which is responsible for the leukocyte activation and migration induced by these cytokines.

5.0 Conclusion

Our studies demonstrate that IL-24 induces myeloid cell migration both in vitro and in vivo.

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References

1. Kunz S, Wolk K, Witte E, Witte K, Doecke WD, Volk HD, Sterry W, Asadullah K, Sabat R. Interleukin (IL)-19, IL-20 and IL-24 are produced by and act on keratinocytes and are distinct from classical ILs. *Exp Dermatol*. 2006; 15:991–1004. [PubMed: 17083366]
2. Commins S, Steinke JW, Borish L. The extended IL-10 superfamily: IL-10, IL-19, IL-20, IL-22, IL-24, IL-26, IL-28, and IL-29. *J Allergy Clin Immunol*. 2008; 121:1108–11. [PubMed: 18405958]
3. Buzas K, Megyeri K. Staphylococci induce the production of melanoma differentiation-associated protein-7/IL-24. *Acta Microbiol Immunol Hung*. 2006; 53:431–40. [PubMed: 17278711]
4. Garn H, Schmidt A, Grau V, Stumpf S, Kaufmann A, Becker M, Gerns D, Siese A. IL-24 is expressed by rat and human macrophages. *Immunobiology*. 2002; 205:321–34. [PubMed: 12182458]
5. Caudell EG, Mumm JB, Poindexter N, Ekmekcioglu S, Mhashilkar AM, Yang XH, Retter MW, Hill P, Chada S, Grimm EA. The protein product of the tumor suppressor gene, melanoma differentiation-associated gene 7, exhibits immunostimulatory activity and is designated IL-24. *J Immunol*. 2002; 168:6041–6. [PubMed: 12055212]
6. Zheng M, Bocangel D, Doneske B, Mhashilkar A, Ramesh R, Hunt KK, Ekmekcioglu S, Sutton RB, Poindexter N, Grimm EA, Chada S. Human interleukin 24 (MDA-7/IL-24) protein kills breast cancer cells via the IL-20 receptor and is antagonized by IL-10. *Cancer Immunol Immunother*. 2007; 56:205–15. [PubMed: 16710719]

7. Nagalakshmi ML, Murphy E, McClanahan T, de Waal Malefyt R. Expression patterns of IL-10 ligand and receptor gene families provide leads for biological characterization. *Int Immunopharmacol.* 2004; 4:577–92. [PubMed: 15120644]
8. Wang M, Liang P. Interleukin-24 and its receptors. *Immunology.* 2005; 114:166–70. [PubMed: 15667561]
9. Sarkar D, Lebedeva IV, Gupta P, Emdad L, Sauane M, Dent P, Curiel DT, Fisher PB. Melanoma differentiation associated gene-7 (mda-7)/IL-24: a 'magic bullet' for cancer therapy? *Expert Opin Biol Ther.* 2007; 7:577–86. [PubMed: 17477796]
10. Fisher PB, Sarkar D, Lebedeva IV, Emdad L, Gupta P, Sauane M, Su ZZ, Grant S, Dent P, Curiel DT, Senzer N, Nemunaitis J. Melanoma differentiation associated gene-7/interleukin-24 (mda-7/IL-24): novel gene therapeutic for metastatic melanoma. *Toxicol Appl Pharmacol.* 2007; 224:300–7. [PubMed: 17208263]
11. Gupta P, Su ZZ, Lebedeva IV, Sarkar D, Sauane M, Emdad L, Bachelor MA, Grant S, Curiel DT, Dent P, Fisher PB. mda-7/IL-24: multifunctional cancer-specific apoptosis-inducing cytokine. *Pharmacol Ther.* 2006; 111:596–628. [PubMed: 16464504]
12. Kotenko SV, Langer JA. Full house: 12 receptors for 27 cytokines. *Int Immunopharmacol.* 2004; 4:593–608. [PubMed: 15120645]
13. Qian W, Liu J, Tong Y, Yan S, Yang C, Yang M, Liu X. Enhanced antitumor activity by a selective conditionally replicating adenovirus combining with MDA-7/interleukin-24 for B-lymphoblastic leukemia via induction of apoptosis. *Leukemia.* 2007
14. Kotenko SV. The family of IL-10-related cytokines and their receptors: related, but to what extent? *Cytokine Growth Factor Rev.* 2002; 13:223–40. [PubMed: 12486876]
15. Sarkar D, Su ZZ, Lebedeva IV, Sauane M, Gopalkrishnan RV, Valerie K, Dent P, Fisher PB. mda-7 (IL-24) Mediates selective apoptosis in human melanoma cells by inducing the coordinated overexpression of the GADD family of genes by means of p38 MAPK. *Proc Natl Acad Sci U S A.* 2002; 99:10054–9. [PubMed: 12114539]
16. Yacoub A, Mitchell C, Hong Y, Gopalkrishnan RV, Su ZZ, Gupta P, Sauane M, Lebedeva IV, Curiel DT, Mahasreshti PJ, Rosenfeld MR, Broaddus WC, James CD, Grant S, Fisher PB, Dent P. MDA-7 regulates cell growth and radiosensitivity in vitro of primary (non-established) human glioma cells. *Cancer Biol Ther.* 2004; 3:739–51. [PubMed: 15197348]
17. Wang CJ, Xue XB, Yi JL, Chen K, Zheng JW, Wang J, Zeng JP, Xu RH. Melanoma differentiation-associated gene-7, MDA-7/IL-24, selectively induces growth suppression, apoptosis in human hepatocellular carcinoma cell line HepG2 by replication-incompetent adenovirus vector. *World J Gastroenterol.* 2006; 12:1774–9. [PubMed: 16586551]
18. Lebedeva IV, Emdad L, Su ZZ, Gupta P, Sauane M, Sarkar D, Staudt MR, Liu SJ, Taher MM, Xiao R, Barral P, Lee SG, Wang D, Vozhilla N, Park ES, Chatman L, Boukerche H, Ramesh R, Inoue S, Chada S, Li R, De Pass AL, Mahasreshti PJ, Dmitriev IP, Curiel DT, Yacoub A, Grant S, Dent P, Senzer N, Nemunaitis JJ, Fisher PB. mda-7/IL-24, novel anticancer cytokine: focus on bystander antitumor, radiosensitization and antiangiogenic properties and overview of the phase I clinical experience (Review). *Int J Oncol.* 2007; 31:985–1007. [PubMed: 17912425]
19. Halliday GM, Le S. Transforming growth factor-beta produced by progressor tumors inhibits, while IL-10 produced by regressor tumors enhances, Langerhans cell migration from skin. *Int Immunol.* 2001; 13:1147–54. [PubMed: 11526095]
20. Hsieh MY, Chen WY, Jiang MJ, Cheng BC, Huang TY, Chang MS. Interleukin-20 promotes angiogenesis in a direct and indirect manner. *Genes Immun.* 2006; 7:234–42. [PubMed: 16511554]
21. Dieu-Nosjean MC, Massacrier C, Vanbervliet B, Fridman WH, Caux C. IL-10 induces CCR6 expression during Langerhans cell development while IL-4 and IFN-gamma suppress it. *J Immunol.* 2001; 167:5594–602. [PubMed: 11698430]
22. Balabanian K, Foussat A, Bouchet-Delbos L, Couderc J, Krzysiek R, Amara A, Baleux F, Portier A, Galanaud P, Emilie D. Interleukin-10 modulates the sensitivity of peritoneal B lymphocytes to chemokines with opposite effects on stromal cell-derived factor-1 and B-lymphocyte chemoattractant. *Blood.* 2002; 99:427–36. [PubMed: 11781221]

23. Musso T, Cappello P, Stornello S, Ravarino D, Caorsi C, Otero K, Novelli F, Badolato R, Giovarelli M. IL-10 enhances CCL2 release and chemotaxis induced by CCL16 in human monocytes. *Int J Immunopathol Pharmacol*. 2005; 18:339–49. [PubMed: 15888256]
24. Ramesh R, Mhashilkar AM, Tanaka F, Saito Y, Branch CD, Sieger K, Mumm JB, Stewart AL, Boquoi A, Dumoutier L, Grimm EA, Renauld JC, Kotenko S, Chada S. Melanoma differentiation-associated gene 7/interleukin (IL)-24 is a novel ligand that regulates angiogenesis via the IL-22 receptor. *Cancer Res*. 2003; 63:5105–13. [PubMed: 12941841]
25. Poindexter NJ, Williams RR, Powis G, Jen E, Caudle AS, Chada S, Grimm EA. IL-24 is expressed during wound repair and inhibits TGFalpha-induced migration and proliferation of keratinocytes. *Exp Dermatol*. 2010; 19:714–22. [PubMed: 20545760]
26. Howard OM, Dong HF, Su SB, Caspi RR, Chen X, Plotz P, Oppenheim JJ. Autoantigens signal through chemokine receptors: uveitis antigens induce CXCR3- and CXCR5-expressing lymphocytes and immature dendritic cells to migrate. *Blood*. 2005; 105:4207–14. [PubMed: 15713799]
27. Clish CB, O'Brien JA, Gronert K, Stahl GL, Petasis NA, Serhan CN. Local and systemic delivery of a stable aspirin-triggered lipoxin prevents neutrophil recruitment in vivo. *Proc Natl Acad Sci U S A*. 1999; 96:8247–52. [PubMed: 10393980]
28. Sironi M, Guglielmotti A, Polentarutti N, Fioretti F, Milanese C, Romano M, Vignini C, Coletta I, Sozzani S, Bernasconi S, Vecchi A, Pinza M, Mantovani A. A small synthetic molecule capable of preferentially inhibiting the production of the CC chemokine monocyte chemoattractant protein-1. *Eur Cytokine Netw*. 1999; 10:437–42. [PubMed: 10477401]
29. Tian YC, Chen YC, Chang CT, Hung CC, Wu MS, Phillips A, Yang CW. Epidermal growth factor and transforming growth factor-beta1 enhance HK-2 cell migration through a synergistic increase of matrix metalloproteinase and sustained activation of ERK signaling pathway. *Exp Cell Res*. 2007; 313:2367–77. [PubMed: 17467690]
30. Wang M, Tan Z, Thomas EK, Liang P. Conservation of the genomic structure and receptor-mediated signaling between human and rat IL-24. *Genes Immun*. 2004; 5:363–70. [PubMed: 15175645]
31. Schaller E, Macfarlane AJ, Rupec RA, Gordon S, McKnight AJ, Pfeffer K. Inactivation of the F4/80 glycoprotein in the mouse germ line. *Mol Cell Biol*. 2002; 22:8035–43. [PubMed: 12391169]
32. He M, Liang P. IL-24 transgenic mice: in vivo evidence of overlapping functions for IL-20, IL-22, and IL-24 in the epidermis. *J Immunol*. 2010; 184:1793–8. [PubMed: 20061404]
33. Ma Y, Chen H, Wang Q, Luo F, Yan J, Zhang XL. IL-24 protects against *Salmonella typhimurium* infection by stimulating early neutrophil Th1 cytokine production, which in turn activates CD8+ T cells. *Eur J Immunol*. 2009; 39:3357–68. [PubMed: 19830736]
34. Trivella DB, Ferreira-Junior JR, Dumoutier L, Renauld JC, Polikarpov I. Structure and function of interleukin-22 and other members of the interleukin-10 family. *Cell Mol Life Sci*. 2010; 67:2909–35. [PubMed: 20454917]
35. Diegelmann J, Beigel F, Zitzmann K, Kaul A, Goke B, Auernhammer CJ, Bartenschlager R, Diepolder HM, Brand S. Comparative Analysis of the Lambda-Interferons IL-28A and IL-29 regarding Their Transcriptome and Their Antiviral Properties against Hepatitis C Virus. *PLoS One*. 2010; 5:e15200. [PubMed: 21170333]
36. Ding Y, Qin L, Zamarin D, Kotenko SV, Pestka S, Moore KW, Bromberg JS. Differential IL-10R1 expression plays a critical role in IL-10-mediated immune regulation. *J Immunol*. 2001; 167:6884–92. [PubMed: 11739506]
37. Liao YC, Liang WG, Chen FW, Hsu JH, Yang JJ, Chang MS. IL-19 induces production of IL-6 and TNF-alpha and results in cell apoptosis through TNF-alpha. *J Immunol*. 2002; 169:4288–97. [PubMed: 12370360]
38. Hsu YH, Li HH, Hsieh MY, Liu MF, Huang KY, Chin LS, Chen PC, Cheng HH, Chang MS. Function of interleukin-20 as a proinflammatory molecule in rheumatoid and experimental arthritis. *Arthritis Rheum*. 2006; 54:2722–33. [PubMed: 16947773]

- IL-24 induces human monocyte and neutrophil migration, in vitro.
- IL-24 attracted CD11b positive murine myeloid cells, in vivo.
- Pertussis toxin, MEK and JAK inhibitors reduced IL-24-induced migration.
- IL-24 activates several nonredundant signaling cascades in immune cells.

Figure 1. IL-24 induced human monocyte and neutrophil migration – In vitro

Human monocytes were assayed for chemotactic activity using a micro-Boyden chamber. Recombinant human IL-24 was acquired from R&D Systems and diluted in chemotaxis media. Chemotactic Index is shown on the y-axis and is relative to spontaneous migration, mean and sem are reported. Migration of human monocytes are shown with right hatched and black bars, while migration of human neutrophils is shown with gray bars. * indicate a p-value < 0.001 relative to spontaneous migration, determined by unpaired T-test. N>6

Figure 2. IL-24 induced leukocyte migration - in vivo

Recombinant IL-24 in HBSS was injected into air pouches formed on the back of C57BL/6 mice. Twenty-four hours later, cells were recovered from the air pouches and counted. Mean cell numbers in millions $\pm$ sem are reported for each treatment condition, with the control being HBSS containing no chemoattractant. * $p=0.01$ student-T two tailed compared to control ** $p=0.003$ student-T two tailed compared to control. $N \geq 4$.

Figure 3. FACS analysis of in vivo recruited leukocytes

A. Mean proportion or percentage of all recruited leukocytes staining positive for both CD45 and CD3 is shown, along with $\pm$ sem, compared to different eliciting agents injected into the air pouch $N \geq 4$. **B.** Representative dot plot graphic of recruited CD45 gated cells stained for CD3 on the x-axis or CD11b on the y-axis. Percentage of CD45 gated cells staining positive for either CD3 or CD11b are reported in the inset. **C.** Mean percentage of recruited CD11b positive cells also staining positive for F4/80 is shown $\pm$ sem, compared to different eliciting agents injected into the air pouch $N \geq 4$. **D.** Representative histograms of CD11b+ cells that are also F4/80 positive. Percentage positive (to the right) or negative (to the left) is shown by inset numbers. The shaded histogram shows an isotype control for F4/80. * $p \leq 0.024$ compared to control HBSS injected into the air pouch

Figure 4. IL-24-induced human monocyte migration can be inhibited

A. Pertussis toxin pretreatment inhibits IL-24 induced monocyte chemotaxis.

Chemotaxis to media, CCL2 or IL-24 is shown in hatched bars. The chemotaxis of monocytes pretreated for 30 minutes with 100ng/ml pertussis toxin is shown in solid black bars. * indicates increases in migration with p-values >0.001 compared to media control # indicates decreases in migration with p-values >0.01 compared to no pertussis toxin at the same concentration of chemoattractant. **B: Pretreatment of monocytes with U0126 inhibits IL-24-induced monocyte chemotaxis.** Human monocytes were pretreated for 30 minutes with 50μM U0126 (MEK inhibitor) prior to being placed in a micro-Boyden chamber. Media alone is shown in white bars. IL-24 is shown with black bars. U0126 treatment is indicated in hatched bars. * indicates increases in migration with p-values >0.001 compared to media control # indicates decreases in migration with p-values > 0.001 compared to no U0126 treatment **C: Pretreatment of monocytes with AG 490 inhibits IL-24-induced monocyte chemotaxis** Human monocytes were incubated with 50 μM AG

490 (JAK inhibitor) for 30 minutes prior to being placed in a chemotaxis chamber. Media is shown with white bars, CCL2 (100 ng/ml) is shown with gray bars, IL-24 is shown with black bars. AG-490 treated cells are shown with hatched bars. * indicates increases in migration with p-values >0.001 compared to media control # indicates decreases in migration with p-values > 0.001 compared to no AG-490 treatment. $N > 6$

Table I
Checkerboard analysis of IL-24 induced monocyte migration

Human monocytes were pretreated with media (0), 1.2 ng/ml, 0.12 ng/ml or 0.001 ng/ml of IL-24 for 30 minutes prior to being placed in a micro Boyden chemotaxis chamber. Across the top of the table are the pretreatment conditions and along the left hand side of the table are the lower well chemoattractant concentrations. Reported in this table are the mean cell number and SEM for each of the conditions. N>3 The mean cell no. per high powered field $\pm$ sem from 3 donors is reported

Upper wells pretreatment/lower wells	1.2 ng/ml	0.12 ng/ml	0.001 ng/ml	0
0 ng/ml	5.9 $\pm$ 1.0 [#]	7.0 $\pm$ 1.0	10.18 $\pm$ 1.5	8.8 $\pm$ 1.1
.001	7.7 $\pm$ 1.0	15.4 $\pm$ 1.6 ^{**}	7.3 $\pm$ 0.7	6.7 $\pm$ 1.2
.12	12.6 $\pm$ 1.8 [*]	18.4 $\pm$ 2.6 ^{**}	20.9 $\pm$ 3.9 ^{**}	18.4 $\pm$ 3.0 ^{**}
1.2	10.4 $\pm$ 1.9	10.4 $\pm$ 2.2	n.d.	12.4 $\pm$ 2.1

[#]
p=0.01 reduced

^{*}
p=0.05 increased

^{**}
P=0.006 increased

n.d = not determined for all donors