The Journal of Immunology

Cutting Edge: Immune Cells as Sources and Targets of the IL-10 Family Members?

Kerstin Wolk, Stefanie Kunz, Khusru Asadullah and Robert Sabat

J Immunol 2002; 168:5397-5402; ;
doi: 10.4049/jimmunol.168.11.5397
http://www.jimmunol.org/content/168/11/5397

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

CUTTING EDGE

Cutting Edge: Immune Cells as Sources and Targets of the IL-10 Family Members?

Kerstin Wolk,* Stefanie Kunz,* Khusru Asadullah,* and Robert Sabat[1]*†

This study investigated the expression of five novel human IL-10-related molecules and their receptors in blood mononuclear cells. IL-19 and IL-20 were found to be preferentially expressed in monocytes. IL-22 and IL-26 (AK155) expression was exclusively detected in T cells, especially upon type 1 polarization, and in NK cells. IL-24 (melanoma differentiation-associated gene 7) expression was restricted to monocytes and T cells. Detection of these molecules in lymphocytes was predominantly linked to cellular activation. Regarding T cells, IL-26 was primarily produced by memory cells, and its expression was independent on costimulation. In contrast to the high expression of receptors for IL-10 homologs in different tissues and cell lines, monocytes and NK, B, and T cells showed clear expression only of IL-10R1, IL-10R2, and IL-20R2. In these cells, IL-20R2 might be part of a still-unknown receptor complex. Therefore, immune cells may represent a major source but a minor target of the novel IL-10 family members. ***The Journal of Immunology*****, 2002, 168: 5397–5402.**

Interleukin-10, discovered more than a decade ago, represents one of the most important immunoregulative cytokines. Among a wide range of actions mediated by this cytokine, it inhibits the inflammatory and the specific T cell response, mainly by affecting monocytic cells (for review see Ref. 1). Recently, five novel human molecules structurally related to the IL-10 have been discovered (2–6). They are IL-19, IL-20, IL-22, AK155, and melanoma differentiation-associated gene (mda)[2]-7. Similar to IL-10, they are secreted α-helical proteins whose amino acid sequences are up to ~30% identical to that of IL-10 and comprise definite positions for cysteine. Interestingly, the encoding genes are located in the human genome in two clusters, one comprising the genes for IL-10, IL-19, IL-20, and mda-7 on chromosome 1q31–32, and another comprising the AK155 and IL-22-encoding genes located on human chromosome 12q15 (3, 7). Like IL-10, all the receptors of the novel molecules known so far belong to the cytokine receptor family type 2 (8). They are mostly transmembrane glycoproteins whose extracellular domains consist of ~210 aa comprising two tandem fibronectin type III domains and having several conserved amino acid positions important for the secondary structure. In general, after ligand binding two particular receptor chains, R1 and accessory R2, are aggregated, forming the final functional receptor complex. Via their heterogeneous intracellular domains they transduce the ligand binding signal preferentially by Janus kinases-STAT pathways. Very recently, it has been discovered that some of the human IL-10 homologs share single receptor chains and even whole receptor complexes (9–12). Taking into account the clear relation between the novel IL-10 homologs and IL-10, the entirety of these six molecules should be considered as (IL-10) family members.

In contrast to the extensively studied IL-10, the knowledge of the biology of the novel IL-10 homologs is still fragmentary. First functional data exist for IL-20, IL-22, and mda-7. Overexpression of IL-20 in transgenic mice induced neonatal lethality, psoriasis-like skin abnormalities, lack of adipose tissue, and elevated apoptosis of thymic lymphocytes (3). IL-22 was suggested to play a role in inflammatory processes through the observation that it induces acute phase reactant production in a hepatoma cell line and in vivo (9). Overexpression of mda-7 via adenoviral gene transfer induced growth inhibition in various tumor types (13). Interestingly, the mda-7 mouse counterpart, called FISP, was postulated to be a Th2-specific protein (14). No function is known for IL-19 and AK155 yet.

A first step in understanding the biological role of the novel molecules is the identification of their cellular sources and their target cell populations. So far, the knowledge about that is rather unsatisfying. The relation of these molecules to IL-10 suggests that they are also immunologically relevant; therefore, this study analyzed the gene expression of the IL-10-related molecules and their receptors in immune cells.

Materials and Methods

Cells

PBMCs from healthy donors were isolated from venous blood as previously described (15). Monocytes, NK cells, B cells, T cells, and T cell subsets were prepared from PBMCs using depletion MACS systems (Miltenyi Biotec, Bergisch Gladbach, Germany) obtaining purities of at least 92% or, in case of double depletion, 85%. Cells were cultured in endotoxin-tested medium as described previously (15). Isolated cells (Figs. 1 and 2) were stimulated or not (controls) with 100 ng/ml LPS from *Escherichia coli* 0127 B8 (monocytes; Sigma-Aldrich, Deisenhofen, Germany), 0.001% (w/v) heat-killed, formalin-fixed *Staphylococcus aureus* cells (B cells; PANSORBIN; Calbiochem-Novabiochem, Bad Soden, Germany), 10 ng/ml IL-2 and 10 ng/ml IL-12 (NK cells; R&D Systems, Wiesbaden-

*Department of Experimental Dermatology, Schering AG, and †Institute of Medical Immunology, Medical School Charité, Humboldt University, Berlin, Germany

Received for publication January 15, 2002. Accepted for publication April 5, 2002.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Address correspondence and reprint requests to Dr. Robert Sabat, Department of Experimental Dermatology, Schering AG, Muellerstrasse 178, D-13342 Berlin, Germany. E-mail address: robert.sabat@schering.de

[2] Abbreviations used in this paper: mda, melanoma differentiation-associated gene; T1, type 1; T2, type 2; FAM, 6-carboxyfluorescein; TAMRA, 6-carboxytetramethylrhodamine.

0022-1767/02/$02.00

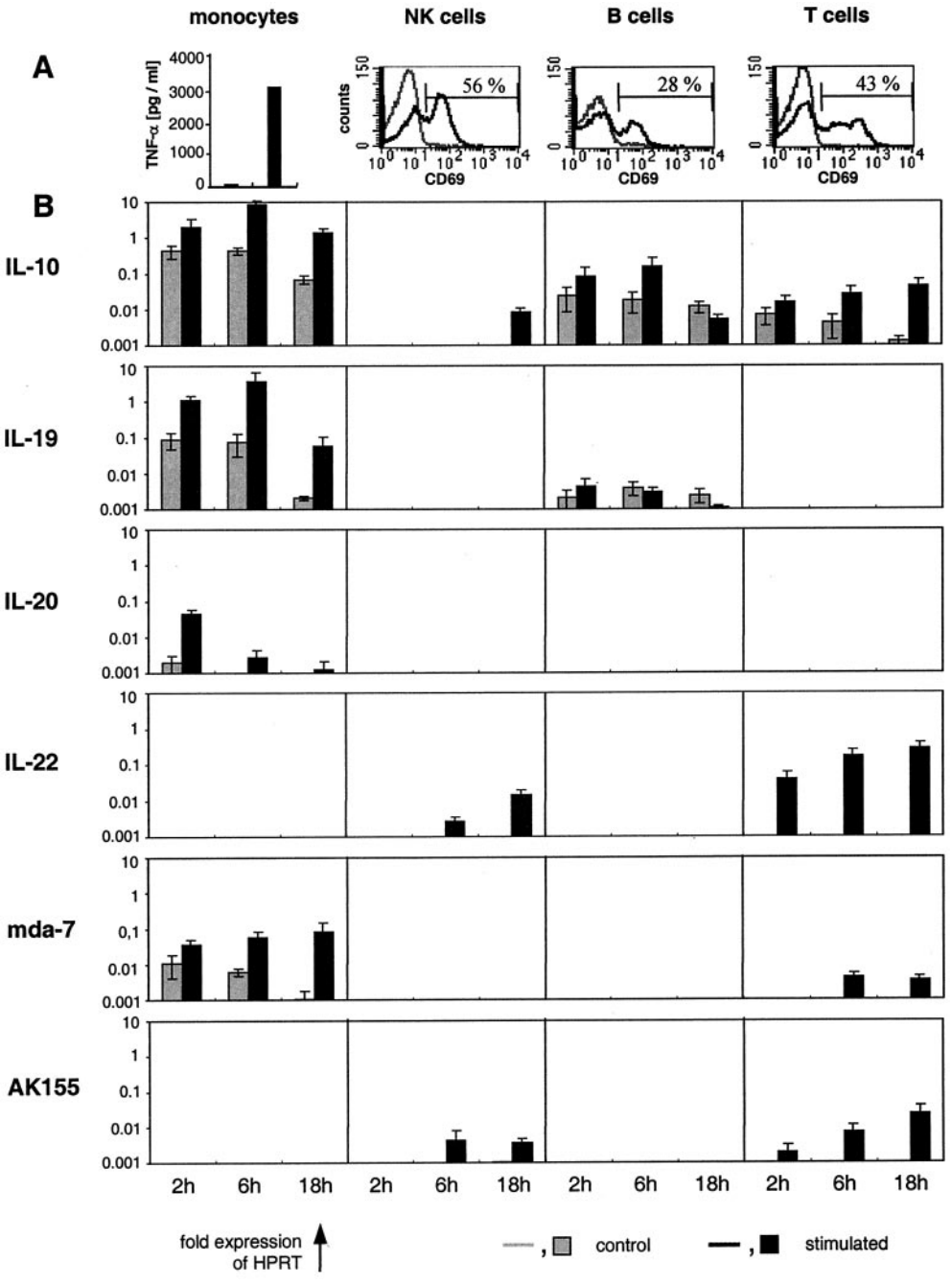

FIGURE 1. Expression of IL-10 family members in blood mononuclear cells. Isolated cells were stimulated or not (controls) with LPS (monocytes), fixed *S. aureus* cells (B cells), IL-2/IL-12 (NK cells), and anti-CD3 mAb (T cells) for 2, 6, and 18 h. *A*, The TNF-α production by monocytes and the CD69 expression on lymphocytes were assessed at 6 h by Immulite and at 18 h by flow cytometry, respectively. Data from one representative experiment are shown. *B*, Gene expression of IL-10 homologs was analyzed by real-time RT-PCR. Expression data relative to those of the housekeeping gene from three independent assays are given as mean ± SEM.

-Nordenstadt, Germany), or anti-CD3 mAb coated on culture vessel (T cells and T cell subpopulations; 1 μg/cm^2; Orthoclone; Cilag, Sulzbach, Germany) for the indicated times. To study the effect of T cell costimulation and functional polarization (Fig. 3), T cells were cultured either in the presence of 5 μg/ml IgG1 and 5 μg/ml IgG2a (controls) or stimulated with anti-CD3 (Cilag) and anti-CD28 mAbs (R&D Systems) coated on culture vessel (1 μg/cm^2 each), in the presence of 5 μg/ml IgG1 and 5 μg/ml IgG2a, 10 ng/ml IL-12 and 5 μg/ml anti-IL-4 mAb, 10 ng/ml IL-4 and 5 μg/ml anti-IFN-γ mAb (all from R&D Systems), or 10 ng/ml IL-10 (PeproTech, Rock Hill, SC) and 10 ng/ml TGF-β1.2 (R&D Systems) for 6, 18, 42, and 66 h. Human EBV-transformed B cells were provided by Dr. N. Babel (Charite, Berlin, Germany). The human pancreatic adenocarcinoma cell line BxPC-3 and the human hepatocyte carcinoma cell line Hep G2 were purchased from European Cell Culture Collection (Salisbury, U.K.). The human keratinocyte cell line, HaCaT, was provided by Dr. N. E. Fusenig (Deutsches Krebsforschungs-Zentrum, Heidelberg, Germany).

Flow cytometry

Assessment of composition of isolated cell populations and confirmation of cellular activation were performed by flow cytometry as previously described (15), with the additional use of the following fluorescence-labeled mAb clones: 13B8.2 (anti-CD4) and B9.11 (anti-CD8) from Coulter Immunotech (Hamburg, Germany) and HI100 (anti-CD45RA) and UCHL1 (anti-CD45RO) from BD PharMingen (Hamburg, Germany).

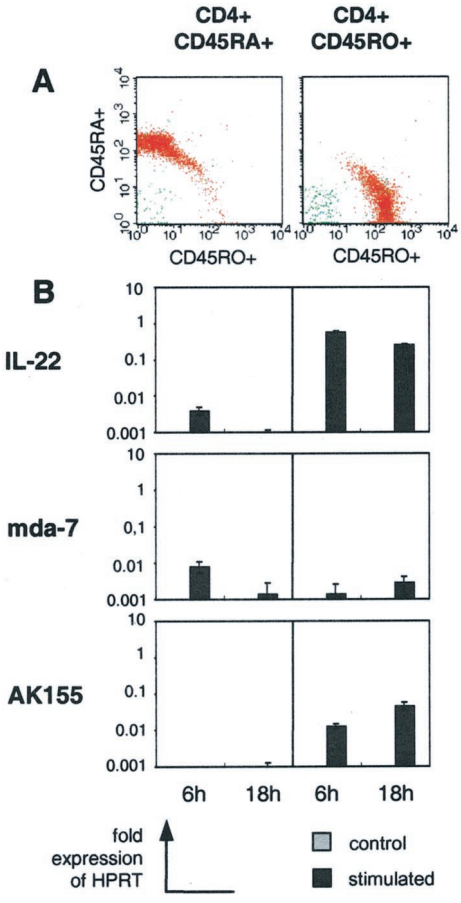

FIGURE 2. Expression of novel members of the IL-10 family in $CD4^+$ naive and memory T cells. Enriched $CD45RA^+$ and $CD45RO^+$ populations of $CD4^+$ T cells were stimulated or not with anti-CD3 mAbs for 6 and 18 h. Gene expression of IL-22, mda-7, and AK155 was analyzed by real-time RT-PCR. *A*, Representative dot plots from flow cytometric analysis show the purity of analyzed cell populations. $CD4^+$ cells are presented in red. *B*, Expression data relative to those of housekeeping gene from two independent experiments are given as mean ± range.

TNF-α quantification

TNF-α concentration in monocyte culture supernatant was measured as previously described (15).

Gene expression analysis

Total cellular RNA from isolated blood cells and different cell lines was prepared as described previously (15). Total RNA from human tissues was obtained from Clontech Laboratories (Heidelberg, Germany). mRNA was reverse transcribed and analyzed by TaqMan PCR as described previously (15). Primers and 6-carboxyfluorescein (FAM)/6-carboxytetramethylrhodamine (TAMRA) double-labeled probes for analyzing IL-10 family members and their receptors as listed in Table I. Additionally, the expression of IFN-γ, IL-4, and TGF-β was analyzed to check initiated T cell polarization. Because preceding experiments demonstrated amplification efficiencies in our system of nearly 1 for all panels, specific gene expression was calculated relative to that of the housekeeping gene hypoxanthine phosphoribosyl-transferase-1. The detection limit for accurate quantification was at 0.001-fold expression of hypoxanthine phosphoribosyl-transferase-1.

Results and Discussion

Given the fact that IL-10 is especially produced by immune cells, we investigated whether the same is true for the other members of the IL-10 family, IL-19, IL-20, IL-22, mda-7, and AK155, and, if so, to what extent. Monocytes, NK cells, B cells, and T cells from the blood of different healthy donors were investigated for their possible constitutive, as well as activation-induced, expression of these molecules. For this purpose, cells were cultured in a kinetic approach in the absence and presence of the typical cell-specific stimuli LPS (monocytes), IL-2/IL-12 (NK cells), fixed *S. aureus* (B cells), and anti-CD3 mAb coated on the culture vessel (T cells), respectively, for 2, 6, and 18 h. The activation state of control and stimulated cells was verified by the absence and presence of high TNF-α production in the monocyte culture and elevated expression of different activation markers, e.g., CD69, on the lymphocyte populations (Fig. 1*A*). Gene expression of the IL-10 family members was analyzed by real-time RT-PCR. IL-10 is known to be produced by various cell types, e.g., activated monocytes, NK cells, B cells, and T cells (for review see Ref. 1). As shown in Fig. 1*B*, IL-10 mRNA could also be detected in resting monocytes, B cells, and T cells. Among the analyzed cells, monocytes appear to be the strongest producers of IL-10, and activation-induced IL-10 mRNA was detected at 2–18 h, peaking at 6 h. In T cells IL-10 expression increased during stimulation time, and in NK cells it was detected at low levels only after 18 h of stimulation. In contrast to IL-10, little is known about the cellular expression of the novel IL-10-related molecules. Using Northern blot analysis, Gallagher et al. (2) recently described IL-19 expression in PBMCs and monocytes becoming apparent after 4 h of LPS stimulation. Our results complement these data, showing clear IL-19 levels already in unstimulated monocytes and an activation-induced expression kinetically very similar to IL-10. Moreover, we can demonstrate the complete absence of IL-19 expression in resting as well as in activated NK and T cells. Very low and irregular expression of IL-19 was also detected in B cells. Although we cannot absolutely exclude the IL-19 presence in these cells due to contamination by monocytes, B cells in principle appear to be able to produce IL-19. In line with the fact that EBV-transformed B cell cDNA served as source for first cloning of IL-19 (2), our studies revealed clear IL-19 mRNA expression in such cells (data not shown). The cellular sources of IL-20 are not known so far. In this work we show that it is produced by monocytes early after stimulation (2 h) and is rapidly down-regulated afterward. This kinetics suggest that IL-20 might be an early proinflammatory cytokine. Interestingly, no other cell population analyzed in this study expressed this molecule. mda-7 was first discovered as a gene induced during terminal differentiation of human melanoma cells in response to IFN-β and the protein kinase C activator mezerein (6). In this work we show that it is also clearly expressed in monocytes and up-regulated in these cells after stimulation at all time points. Slight and delayed mda-7 expression was also seen in our study in activated T cells. IL-22 was originally identified in the murine system as a molecule differentially expressed in IL-9-treated murine T lymphoma cells, and its mRNA expression was also reported in various organs in the mouse after LPS injection (4, 9). In this study we show that IL-22 expression is specific for activated T cells and, at lower levels, NK cells in which it increases with time. A similar expression pattern was detected for AK155 expression. Until now, only one publication about AK155 exists describing the expression in virus-transformed T cells as detected by Northern blot assay and, using more sensitive RT-PCR, also in normal T cells and PBMC. Taken together, in contrast to IL-10 being expressed in monocytes, NK cells, B cells, and T cells, the production of the novel IL-10 homologs is restricted to special populations where it is up-regulated after cellular activation. Regarding the expression pattern, three groups may be distinguished: those that are preferentially expressed in monocytes (IL-19, IL-20), those that are restricted to (activated) T cells and NK cells (IL-22, AK155), and, finally, the mda-7, which is expressed in monocytes and T cells.

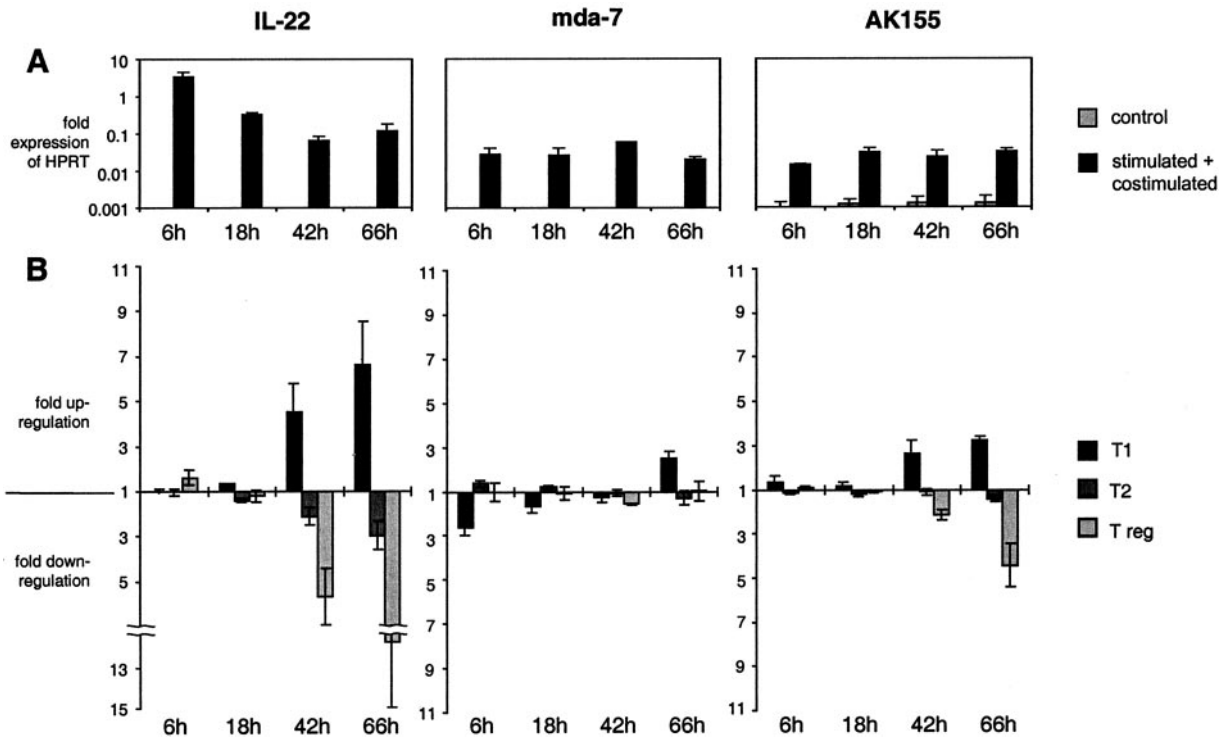

FIGURE 3. Modulation of the T cell expression of novel IL-10 family members by costimulation and functional polarization. T cells were stimulated with anti-CD3 and anti-CD28 mAbs in the presence of IL-12/anti-IL-4 mAb (polarization toward T1), IL-4/anti-IFN-γ mAb (polarization toward T2), IL-10/TGF-β1.2 (polarization toward regulatory T cells), or isotypic control mAbs, or cultured without stimulation in the presence of isotypic control mAbs for 6, 18, 42, and 66 h. Gene expression of IL-10 homologs was analyzed by real-time RT-PCR. *A*, Expression in nonstimulated and stimulated nonpolarized cells is shown as relative to that of the housekeeping gene. *B*, Expression in stimulated polarized cells relative to stimulated nonpolarized cells is shown. Data from three independent assays are given as mean ± SEM.

As already demonstrated, T cells are producers of IL-22, mda-7, and AK155 after TCR engagement, as mimicked by anti-CD3 mAb exposure. When asking for the contribution of the $CD4^+$ and the $CD8^+$ subpopulations to this production we found that $CD4^+$ cells expressed larger amounts of IL-22 and AK155, and marginally more mda-7 than did $CD8^+$ (data not shown). Among $CD4^+$ cells, naive and memory subsets can be distinguished by the presence of CD45RA and CD45RO expression, respectively. As shown in Fig. 2, IL-22 was preferentially produced by activated memory cells. mda-7 was expressed in both activated naive and memory cells, although the kinetics of expression in these subsets were different. AK155 was exclusively expressed in activated memory cells.

We further asked whether costimulation would modulate anti-CD3 mAb-induced production of IL-22, mda-7, and AK155. Cells were cultured on anti-CD3 mAb/anti-CD28 mAb-coated vessels for 6 and 18 h as above, and additionally for 42 and 66 h (Fig. 3*A*). The impact of costimulation on the expression of IL-22, mda-7, and AK155 correlated with the contribution of $CD4^+CD45RA^+$ T cells to the production of these molecules. Compared with anti-CD3 mAb stimulation alone, costimulation clearly increased the extent of IL-22 (19-fold at 6 h and no modulation at 18 h) and mda-7 (~6-fold at 6 h and ~8-fold at 18 h) mRNA. AK155 expression did not clearly increase upon costimulation (<2-fold at 6 h and no difference at 18 h).

Among T cells, one can differentiate between distinct subsets based on their cytokine profile and their functional activities. Type 1 (T1) T cells produce IFN-γ and TNF-α and mediate the cellular immunity. Type 2 (T2) T cells secrete IL-4, IL-5, and IL-13, which in turn regulate the humoral immunity. It has been shown that IL-12 drives polarization toward T1 in a STAT4-dependent manner, and IL-4 STAT6-dependently drives polarization toward T2. Furthermore, the presence of IL-10 and/or TGF-β upon T cell stimulation has been shown to generate a phenotype of regulatory T cells able to counterregulate the activity of T cells (1). We asked whether polarization of T cells toward such subsets would modulate their expression of IL-10 homologs. T cells were activated as in Fig. 3*A* but in the presence of IL-12/neutralizing anti-IL-4 mAb, IL-4/neutralizing anti-IFN-γ mAb, IL-10/TGF-β, or isotypic control mAbs. Fig. 3*B* shows the specific expressions relative to those of nonpolarized T cells. Again, no expression of IL-19 and IL-20 was detected under any conditions tested in these experiments (data not shown). Initiation of T1 polarization enhances the expression of IL-22 and, to a lesser extent, of AK155 at later time points (42 and 66 h). The presence of T2 milieu led to slight reduction of IL-22 expression at 42 and 66 h. Initiation of polarization toward the regulatory phenotype induced massive and slight reduction of IL-22 and AK155 expression, respectively. Therefore, IL-22 and AK155 may represent typical T1 mediators. The murine counterpart of mda-7 has been detected in CD4-positive T2 and also nonpolarized cells in an IL-4-dependent manner (14). Our data show that the expression of mda-7 in the human system seems to be distinctly regulated. At an early time point (6 h) it was down-regulated in cells under T1 milieu and slightly up-regulated in cells under T2 milieu. At 66 h, exposure to T1 milieu increased this production. A distinct regulation of mda-7 in the human system is also underlined by the observation of absent mda-7 expression in LPS-stimulated murine macrophage-like RAW 264.7 cells (14), which is not in line with our data obtained with human monocytes (Fig. 1*B*). Taken together, the novel members of the IL-10 family are essentially

Table I. *Panels for quantitative RT-PCR*

Gene	Oligonucleotide Sequences	Concentration (nM)	Amplification product (bp)
IL-10	5′-GGCAACCTGCCTAACATGCTT-3′ (exon 1)	900	
	5′-CAAGTTGTCCAGCTGATCCTTCAT-3′ (exon 2)	50	90
	FAM-5′-AAAGAAAGTCTTCACTCTGCTGAAGGCATCTCG-3′-TAMRA	200	
IL-19	5′-CATGCACCATATAGAAGAGAGTTTCC-3′ (exon 3)	300	
	5′-TGGACAGGATAGTGACATTTGGG-3′ (exon 4)	300	86
	FAM-5′-AGAAATCAAAAGAGCCATCCAAGCTAAGGACAC-3′-TAMRA	200	
IL-20	5′-GGAGGACTGAGTCTTTGCAAGAC-3′ (exon 2)	900	
	5′-CCGGAGAGTATAATGGTCAGGG-3′ (exon 3)	900	125
	FAM-5′-CAAAGCCTGCGAATCGATGCTGC-3′-TAMRA	200	
IL-22	5′-ACAACACAGACGTTCGTCTCATTG-3′ (exon 2)	300	
	5′-GAACAGCACTTCTTCAAGGGTGA-3′ (exon 3)	300	113
	FAM-5′-TTCCACGGAGTCAGTATGAGTGAGCGCT-3′-TAMRA	200	
mda-7	5′-TTTCAACAGAGGCTGCAAAGC-3′ (exon 2)	900	
	5′-GCACAACCATCTGCATTTGAGA-3′ (exon 3)	900	91
	FAM-5′-ACTTTAGCCAGACCCTTCTGCCCTCCTTT-3′-TAMRA	200	
AK155	5′-GACTTTCATAGCCTTAGGCAGAAATT-3′ (exon 3)	900	
	5′-CATCCTGGTAATGGATTTCATCTCT-3′ (exon 4)	900	84
	FAM-5′-AGCCACTGTATTTCCTGTGCTTCATCAGCT-3′-TAMRA	200	
IL-10R1	5′-GCATCTTCAGTCACTTCCGAGAG-3′ (exon 4)	900	
	5′-ATGGTTTCACCTGGACACAGAAC-3′ (exon 5)	900	147
	FAM-5′-ATGAGATTGCCATTCGCAAGGTGCC-3′-TAMRA	200	
IL-10R2	5′-GAGTGAGCCTGTCTGTGAGCAA-3′ (exon 5)	300	
	5′-TGACGGCCACCATCCAG-3′ (exon 6)	900	65
	FAM-5′-CAACCCATGACGAAACGGTCCCCT-3′-TAMRA	200	
IL-20R1	5′-TGTTGCTCCTGGCGG C-3′ (exon 1)	900	
	5′-CAAACATCACCTTCTTATCCATCAA-3′ (exon 2)	300	91
	5′-CCCTGTGTCTCTGGTGGTTTGCCTAAACCT-3′-TAMRA	200	
IL-20R2	5′-TGTTGCCCCGTGGTGGT-3′ (exon 6)	300	
	5′-CTCCTCCCTTCTGCAGCTGAT-3′ (exon 7)	300	81
	FAM-5′-CTCCCAGACACCTTGAAAATAACCAATTCACC-3′-TAMRA	200	
IL-22R1	5′-CCTGAGCTACAGATATGTCACCAAG-3′ (exon 6)	300	
	5′-GGCTGGAAAGTCAGGACTCG-3′ (exon 7)	300	78
	FAM-5′-ACCTC CCAAC TCCCT GAACG TCCAG-3′-TAMRA	200	
HPRT-1	5′-GACTTTGCTTTCCTTGGTCAGG-3′ (exon 6)	300	
	5′-AGTCTGGCTTATATCCAACACTTCG-3′ (exon 7)	300	101
	FAM-5′-TTTCACCAGCAAGCTTGCGACCTTGA-3′-TAMRA	200	

produced by blood immune cells. In contrast, our preliminary studies demonstrated only minor expression of these molecules among a wide range of human tissues (data not shown). Regarding the structural features and expression patterns, mda-7 and AK155 resemble the other members of the IL-10 family, thus advocating the renaming of these molecules into IL-24 and IL-26, respectively.

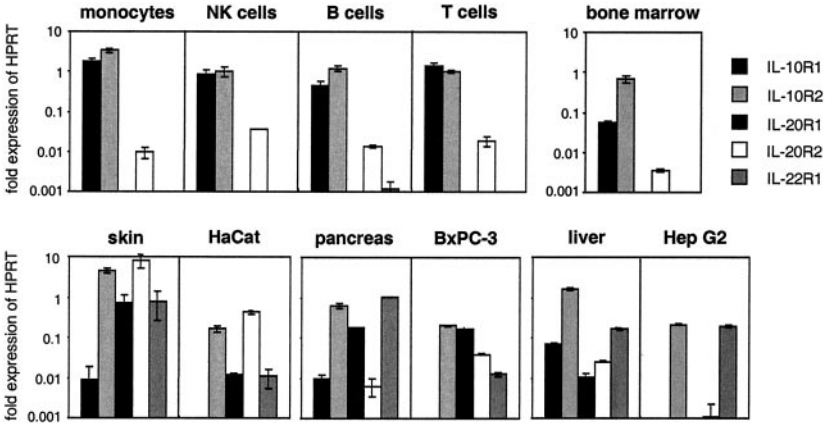

FIGURE 4. Expression of receptors for the IL-10 family members in blood immune cells compared with selected human tissues and their representative cell populations. Freshly isolated blood monocytes, NK cells, B cells, T cells, human tissues, and cell lines were analyzed by real-time PCR for the expression of the receptors for the IL-10 family members. Data from three (primary immune cells) or two (tissues and cell lines) independent assays are given as mean ± SEM (primary immune cells) or mean ± range (tissues and cell lines).

We then asked for the targets of IL-10 family members among immune cells. For this purpose, we analyzed the expression of receptor chains known to function as subunits in its receptor complexes, by quantitative real-time PCR. Three R1 type chains for IL-10 and related molecules, IL-10R1, IL-20R1, and IL-22R1, and two R2 type chains, IL-10R2 and IL-20R2, are known so far. R1 chains can associate with R2 chains to form functional receptors as follows: IL-10R1/IL-10R2 mediate effects of IL-10; IL-20R1/IL-20R2 mediate effects of IL-19, IL-20, and mda-7; IL-22R1/IL-10R2 mediate effects of IL-22; and IL-22R1/IL-20R2 mediate effects of IL-20 and mda-7. Whether IL-10R1/IL-20R2 or IL-20R1/IL-10R2 can form functional receptors is unknown so far. No receptor has been identified for AK155 so far, but it may be assumed that it is within the same receptor family. As demonstrated in Fig. 4, IL-10R1 and IL-10R2 were highly expressed by monocytes, NK cells, B cells, and T cells. Among them, monocytes show the strongest expression of these receptors, suggesting highest sensitivity of these cells toward IL-10. All cell populations also express IL-20R2, though at lower levels. Monocytes, NK cells, and T cells do not express any of the known partner chains of IL-20R2 (neither IL-20R1 nor IL-22R1). Therefore, the isolated expression of IL-20R2 suggests that it may associate with the IL-10R1 or a still unknown R1 chain to confer sensitivity toward another ligand (e.g., AK155). In B cells, expression of IL-22R1 was additionally detected at levels near the detection limit of the used method. Our further data suggest that no additional chain is expressed in the analyzed cell populations upon stimulation for 6 h as described in Fig. 1 or in T cells after 42 h under polarizing conditions as described in Fig. 3 (data not shown). The absent detection of IL-22R1 expression in T cells does not support IL-22 effects on these cells (10). All in all, these data demonstrate a minimal expression of known receptors for the novel IL-10 homologs in immune cells. This suggests that there exist major targets in cells other than these cells. In fact, the analysis of different human tissues and their representative cell populations revealed high specific expressions of different receptors in these samples (Fig. 4).

In summary, this is the first study that investigated the gene expression of the novel members of the IL-10 family and their receptors in a systematic manner in primary immune cells. Our data point out that immune cells exhibit a differential expression of the IL-10 homologs but seem not to be the major target cell populations of these molecules. This is in contrast to the IL-10 that is produced by and acts on all monocytes, NK cells, B cells, and T cells.

Acknowledgments

We thank Dr. J.-C. Renauld (Brussels, Belgium) for making available the IL-20R2 cDNA sequence, Dr. N. Babel for kindly providing EBV-transformed B cells, and Dr. N. E. Fusenig for providing the HaCaT cells. Special thanks are due to K. Folgnand, A. Häussler-Quade, S. Zeinert, and R. Wieseke for technical assistance.

References

1. Moore, K. W., R. de Waal Malefyt, R. L. Coffman, and A. O'Garra. 2001. Interleukin-10 and the interleukin-10 receptor. *Annu. Rev. Immunol. 19:683.*
2. Gallagher, G., H. Dickensheets, J. Eskdale, L. S. Izotova, O. V. Mirochnitchenko, J. D. Peat, N. Vazquez, S. Pestka, R. P. Donnelly, and S. V. Kotenko. 2000. Cloning, expression and initial characterization of interleukin-19 (IL-19), a novel homologue of human interleukin-10 (IL-10). *Genes Immun. 1:442.*
3. Blumberg, H., D. Conklin, W. F. Xu, A. Grossmann, T. Brender, S. Carollo, M. Eagan, D. Foster, B. A. Haldeman, A. Hammond, et al. 2001. Interleukin 20: discovery, receptor identification, and role in epidermal function. *Cell 104:9.*
4. Dumoutier, L., J. Louahed, and J. C. Renauld. 2000. Cloning and characterization of IL-10-related T cell-derived inducible factor (IL-TIF), a novel cytokine structurally related to IL-10 and inducible by IL-9. *J. Immunol. 164:1814.*
5. Knappe, A., S. Hor, S. Wittmann, and H. Fickenscher. 2000. Induction of a novel cellular homolog of interleukin-10, AK155, by transformation of T lymphocytes with herpesvirus saimiri. *J. Virol. 74:3881.*
6. Jiang, H., J. J. Lin, Z. Z. Su, N. I. Goldstein, and P. B. Fisher. 1995. Subtraction hybridization identifies a novel melanoma differentiation associated gene, mda-7, modulated during human melanoma differentiation, growth and progression. *Oncogene 11:2477.*
7. Dumoutier, L., E. Van Roost, G. Ameye, L. Michaux, and J. C. Renauld. 2000. IL-TIF/IL-22: genomic organization and mapping of the human and mouse genes. *Genes Immun. 1:488.*
8. Kotenko, S. V., and S. Pestka. 2000. Jak-Stat signal transduction pathway through the eyes of cytokine class II receptor complexes. *Oncogene 19:2557.*
9. Dumoutier, L., E. Van Roost, D. Colau, and J. C. Renauld. 2000. Human interleukin-10-related T cell-derived inducible factor: molecular cloning and functional characterization as an hepatocyte-stimulating factor. *Proc. Natl. Acad. Sci. USA 97:10144.*
10. Xie, M. H., S. Aggarwal, W. H. Ho, J. Foster, Z. Zhang, J. Stinson, W. I. Wood, A. D. Goddard, and A. L. Gurney. 2000. Interleukin (IL)-22, a novel human cytokine that signals through the interferon receptor-related proteins CRF2–4 and IL-22R. *J. Biol. Chem. 275:31335.*
11. Kotenko, S. V., L. S. Izotova, O. V. Mirochnitchenko, E. Esterova, H. Dickensheets, R. P. Donnelly, and S. Pestka. 2001. Identification of the functional interleukin-22 (IL-22) receptor complex: the IL-10R2 chain (IL-10Rβ) is a common chain of both the IL-10 and IL-22 (IL-10-related T cell-derived inducible factor, IL-TIF) receptor complexes. *J. Biol. Chem. 276:2725.*
12. Dumoutier, L., C. Leemans, D. Lejeune, S. V. Kotenko, and J. C. Renauld. 2001. Cutting edge: STAT activation by IL-19, IL-20, and mda-7 through IL-20 receptor complexes of two types. *J. Immunol. 167:3545.*
13. Jiang, H., Z. Z. Su, J. J. Lin, N. I. Goldstein, C. S. Young, and P. B. Fisher. 1996. The melanoma differentiation associated gene mda-7 suppresses cancer cell growth. *Proc. Natl. Acad. Sci. USA 93:9160.*
14. Schaefer, G., C. Venkataraman, and U. Schindler. 2001. Cutting edge: FISP (IL-4-induced secreted protein), a novel cytokine-like molecule secreted by Th2 cells. *J. Immunol. 166:5859.*
15. Gruenberg, B. H., A. Schoenemeyer, B. Weiss, L. Toschi, S. Kunz, K. Wolk, K. Asadullah, and R. Sabat. 2001. A novel, soluble homologue of the human IL-10 receptor with preferential expression in placenta. *Genes Immun. 2:329.*