Chapter 9
Cytokine Species-Specificity and Humanized Mice

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9.1 Introduction

Cytokines can be defined as small, almost always soluble (although some can also be membrane-bound) proteins, produced to regulate immune responses and body functions often associated with immunity. They often, but not always, are synthetized by immune cells, act at very low concentrations due to their high affinity for their receptor(s) and exert their influence mostly at short range. Importantly, some of them can also be made, under specific circumstances, by cells other than leukocytes. As such they can be compared to hormones, with the main difference being that the cells producing cytokines are generally not part of specific, organized organs or glands, but have a tendency to be disseminated throughout the body.

Since cytokines play a key role in the regulation of immune responses and the interactions between the immune system and other organs (for example the neural system or the liver), it is critical that they be carefully considered in the development of animal models for any disease involving the immune system in general and HIV infection in particular. While in most cases the cytokines' general structure, function and receptors are maintained between humans and mice, there are some exceptions. For example, mice have a specific IL3R-β chain ($\beta_{IL\text{-}3}$), in addition to the common IL-3R-β chain (β_c), which is shared between IL-3, IL-5 and GM-CSF and is the only β-chain in humans [1]. Despite this general conservation between humans and mice, one can expect that in some cases mouse cytokines will not activate human cells and vice versa (i.e., there is not cross-reaction between cytokines of the two species; in other words, the cytokines are species specific). It should be noted that species specificity does not need to be absolute but could be quantitative in that a much higher concentration of the cytokine is required when from a different species. For example, about a thousand times more murine IL-15 is required to activate human cells compared to human IL-15 [2].

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When the cells expressing the cytokine and the cells reacting to the cytokine (i.e., expressing the receptor for the cytokine) have the same origin in a humanized mouse, this does not matter a great deal. However, if the cells expressing the receptor for the cytokine and the cells producing the cytokine are of different origins in the humanized mouse, this becomes very important. While the limitations resulting from the lack of cytokine cross-reactivity between species have been identified as a shortcoming in humanized mice [3], the full implications have not been explored systematically. Here, we address this issue and suggest a way to better identify cytokines more likely to be species specific: based on amino acid identity between mice and humans.

9.2 Humanized Mice

In order to understand the implications of cytokine species specificity in the context of mouse models for HIV, it is essential to briefly consider the basic properties and ways in which mice can be 'humanized'. A basic prerequisite for a mouse model for HIV research is the ability of HIV to infect cells present in the animal. In addition, in as far as possible, a functional human-like immune system must be present in the mouse model. This can, to various degrees, be achieved by creating 'humanized mice', which were defined by Shultz et al as 'immuno-deficient mice that have been engrafted with human primary haematopoietic cells and tissues that generate a functional human immune system' [4]. Basic requirements for the generation of humanized mice are that the transferred human cells must not be rejected, must be located in their normal environment and supported by that environment [5]. We will consider both the source of human immune cells and different types of immunosuppressed mice in the context of species-specific cytokines.

9.2.1 Sources of Human Immune Cells

In their simplest form, human immune cells can be provided by peripheral blood lymphocytes (PBL) [4, 5]. However, the transferred lymphocytes will unleash an immune response towards the mouse host cells, resulting in a xenogeneic graft-versus-host response, which will interfere with subsequent studies. In addition, because the mouse cells do not express the human leukocyte antigen (HLA) molecules, these cells cannot present antigen to the mature human T cells. To address these issues it is possible to repopulate the mouse immune system with human cells by transferring human hematopoietic stem cells, bone marrow cells or peripheral blood following G-CSF treatment. As a result, human precursor cells, including precursor T cells, will develop. These human precursor T cells will migrate to the mouse thymus to complete their differentiation. Hence, they will encounter mouse major histocompatibility complex (MHC) within the thymus. Therefore, the human

T cells are educated to the mouse MHC background and not the HLA background. Indeed, positive and negative selection within the thymus will result in these human T cells only recognizing antigen presented in the context of the mouse MHC. As a result, only mouse cells can now present antigen to the human T cells. Thus in such a model, mouse cytokines produced by the mouse antigen-presenting cells (APC) will need to cross-react with human cells as only mouse cells will be able to activate the human T cells. Conversely, human cytokines produced by the T cells will need to cross-react with mouse APC to allow the activation of the mouse APC by cytokines produced by the human T cells.

To resolve this issue, coimplantation of human fetal liver and thymus under the kidney capsule can be performed. This transplantation results in the generation of HLA-restricted human T cells. However, there is still a relatively poor human immune system, primarily because only low levels of human immune cells are present. To circumvent this problem, human bone marrow can be transferred, in addition to liver and thymus resulting in the bone marrow, liver, thymus (BLT) model [4]. While the human immune system is much more functional in the BLT model, vaccination studies are still limited because IgM antibodies are primarily produced, with only limited class switching occurring, suggesting that immunoregulatory mechanisms are incomplete. In such a model, cross-reactivity between mouse and human cytokines is particularly important for the correct development of the human cells within the 'mouse organ scaffold' (except for the human thymus).

9.2.2 Sources of Immunosuppressed Mice

The recipient mice need to be severely immunosuppressed in order to accept the xenogeneic graft. This immunosuppressed state can be achieved in several different ways. Initially mice were discovered with a mutation in the protein-kinase, DNA-activated, catalytic polypeptide (Prkdc), which resulted in severe combined immunodeficiency (SCID). While these mice were immunodeficient because of their inability to recombine the T cell receptor and immunoglobulin genes, later in life some recombination event could occur, resulting in leakage [6]. Subsequently, a similar phenotype was obtained by targeted mutation of the recombination-activating gene 1 (Rag1) and Rag2 loci, circumventing the issue of leakiness. However, these mice still have a mouse innate immune system including mouse natural killer (NK) T cells, mouse macrophages, which limit the engraftment of human cells. As such, the mouse innate immune cells will produce cytokines, which depending on the type, will or will not activate human cells. To overcome this, SCID mice can be crossed with nonobese diabetic (NOD) mice resulting in NOD–SCID mice with additional, but not absolute, deficiencies in innate immune cell types [6]. By crossing these mice with mice deficient for the IL-2 receptor γ-chain (IL-2Rγ) further improvements in acceptance of human cells were obtained. The IL-2Rγ is needed for signal transduction through the high-affinity receptors for IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21. Hence, targeted mutations at the IL2rg locus coding for the

IL-2Rγ resulted in complete NK T cells impairment [4, 6]. Recent additional improvements include the transgenic expression of HLA genes and knockout of the mouse MHC genes. The idea is that in these mice the human T cells are educated in the mouse thymus but against HLA molecules expressed by the transgene. The approach would result in a more effective education of human T cell in the mouse without requiring a more complicated transplantation of human thymus cells. Indeed theoretically in these mice, T cells will be educated by the mouse thymus, hence using mouse cytokines.

9.2.3 Expressing Human Cytokines in Immuno-Deficient Mice

In an attempt to promote the development of the transferred human immune system, human cytokines have been overexpressed in the mouse under the form of 'knock-ins'. This is particularly important when the mouse cytokine cannot activate human cells. For example, IL-3 and GM-CSF do not cross-react between mouse and human [5]. It is therefore not surprising that knocking-in the genes for human IL-3 and human GM-CSF into immunodeficient mice increases the number of functional human macrophages in these humanized mice [4]. Another example consists of transiently expressing human cytokines (IL-15 and Flt-3/Flk-2 ligand) in the humanized mouse in order to improve the development of human NK cell populations [7]. While IL-15 has been reported to cross-react between humans and mice [5], mouse IL-15 is about three orders of magnitude less effective on human cells compared to human IL-15 [2] providing an explanation for this result. The case of thrombopoietin is interesting because this cytokine is cross-reactive between mice and humans [5]. As a result, one would expect that the mouse cytokine would be able to sustain the human cells. Nevertheless, here again knocking-in the gene for thrombopoetin into immunodeficient mice prior to reconstitution with human cells results in increased human myelopoiesis and ability for human stem cell renewal while decreasing lymphopoeisis. At the same time, it also results in thrombocytopenia and decreased hematopoietic stem cells in the mouse host [4, 8]. Thus, while this approach leads to some improvements, at the same time it creates a new set of issues and complications. Also, this approach will always be limited to a relatively small number of knocked-in human cytokines. Therefore, despite substantial progress it remains that humanized mice do not possess a complete and fully functional human immune system.

Even in the best-case scenario if a complete and functional human immune system can be created in a mouse, the resulting mouse model will still have a human immune system while at the same time retaining mouse tissues for other organs. Therefore species-specific cytokines produced by these transferred human cells and their (differentiated) progenitors will be able to affect only these human cells and not the mouse cells from any other organ. Conversely, species-specific cytokines made by mouse cells in the majority of mouse organs cannot affect the transferred human cells. Hence, the implications of this coexistence of two parallel organ systems need to be considered in detail.

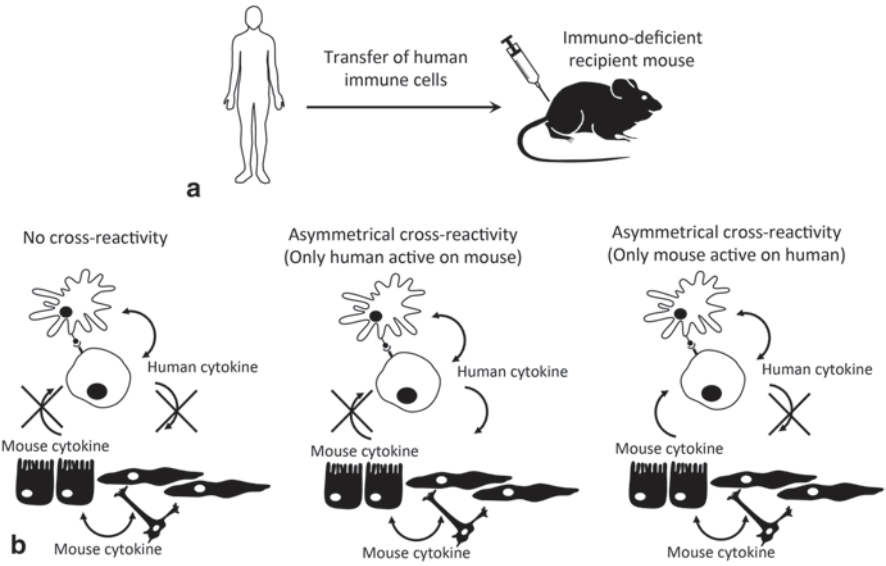

Fig. 9.1 Schematic representation of the effect of species-specific cytokines produced by human leukocytes and mouse nonleukocytes in humanized mice. **a** Humanized mice contain human immune cells that have been transferred into an immunodeficient mouse. While in many cases the human immune system is not completely functional, even a functional immune system will be deficient in its interactions with the other organs of the host mouse. **b** For species-specific cytokines, the human immune system will not be able to interact with a wide variety of cells of the mouse host and vice versa. Asymmetrical cross-reactivity in which the human cytokine will activate mouse cells (but not the reverse) will allow for the human immune system to influence the mouse organs. However, mouse cytokines will not be in a position to influence the human immune system. This often results in lack of complete establishment of a functional human immune system in the mouse host. Asymmetrical cross-reactivity whereby the mouse cytokine can activate the human immune system but not reverse will lead to the lack of key physiological effects of the immune system on the affected organs of the mouse.

9.3 Cytokines as Messengers Between the Immune System and Other Organs

Cytokines are produced mainly by leukocytes and by and large act on leukocytes. Thus in the ideal situation, when all the leukocytes in a mouse model of HIV are replaced by human leukocytes (Fig. 9.1a), correct communication between these cells should occur, irrespective of cytokine species specificity (Fig. 9.1b). However, cytokines can also affect nonleukocytes and regulate other body functions, such as for example, the effects they have on the neural system or on hepatocytes during acute phase responses. In a similar way, but outside of the scope of this review, leukocytes also interact with, for example, endothelial cells during transmigration and species specificity of the ligands and receptors of the human cells migrating through mouse tissues will be important in the way immune cells are able to reach their destination.

If the human cytokines produced by the transferred human leukocytes are species-specific, these cytokines will not perform their nonimmune function (Fig. 9.1b). For example, it is well documented that tumor necrosis factor (TNF) and interferon (IFN)-γ both induce fever and cause pain. However, human IFN-γ is unable to bind the murine receptor [9], while human TNF-α can activate mouse cells [10] possibly resulting in very different outcomes in a humanized mouse depending on which cytokine is responsible for the physiological observation. Thus, due to redundancy in the cytokine network and cross-reactivity of certain but not all cytokines, it is possible that a function is observed but that the underlying mechanism and possibly also the associated feedback loops might be different in humanized mice compared to humans. Conversely, if mouse-derived cells produce species-specific cytokines, these will not be able to affect the human leukocytes, so that mouse organs are impaired in conveying a signal back to the human immune system (Fig. 9.1b).

Thus, for species-specific cytokines we can expect that there would be a disconnect between the leukocyte-mediated functions of the cytokines and the functions of the cytokine mediated by other cells. The same argument can be put forward when nonimmune cells produce the cytokine. These cytokines are produced by host mouse cells and will affect other mouse cells more than they would affect the transferred human leukocytes. Thus here also, there would be a discontinuity between the origin of the cytokine and its function on different cell types (leukocytes vs. all other cell types).

9.3.1 Cytokines Produced by Multiple Cell Types and Interacting with (Multiple) Receptors on Different Cells

In addition, an interesting but complicated and artificial situation arises when a cytokine is produced by more than one cell type and some of these cell types happen to be part of the transferred human cells (and their progenitors), while others are of mouse origin. Indeed, for species-specific cytokines, this situation would result in two different sources of the cytokine, each being able to only affect cells of their own species.

The situation can become even more artificial if there is asymmetrical cross-reactivity (i.e. if the cytokine from one species, say humans, can cross-react on mouse cells, but the mouse cytokine cannot activate human cells). Indeed, under these conditions there are two sources of cytokines with different effects. The human cytokine becomes the ‘super cytokine’, able to activate all cells, while the homologous mouse cytokine is the ‘poor relative’, only able to affect the mouse and not the human cells (Fig. 9.1b). One such complex example is IL-6, which is produced by a wide range of cells including not only leukocytes such as macrophages, T cells, B cells and granulocytes; but also by smooth muscle cells, chondroblasts, osteoblasts and fibroblasts. In a humanized mouse, the IL-6 produced by white blood cells is human and since human IL-6 activates both human and mouse cells [5], it can be considered a ‘super cytokine’. However, at the same time the mouse

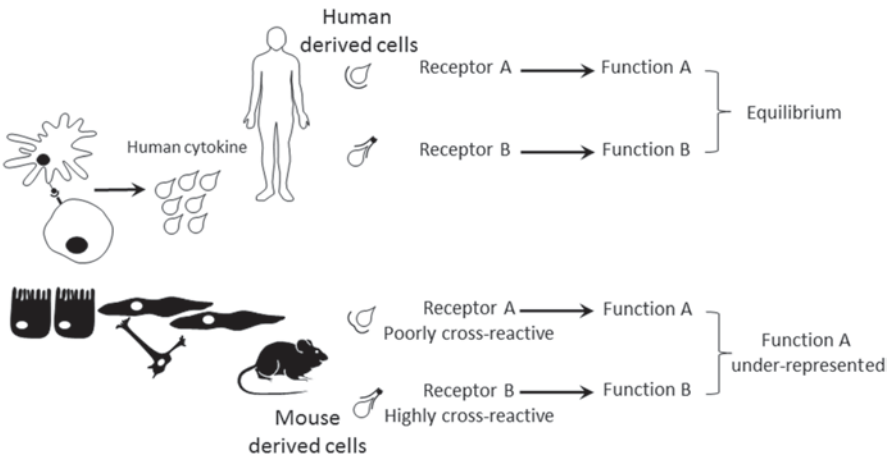

Fig. 9.2 Schematic representation of the effect of one cytokine interacting with several receptors, each having a different degree of cross-reactivity for the cytokine. The human cytokine is produced and will interact optimally with each of its receptors on human cells. When interacting with the mouse cytokine receptors it is likely that the degree of cross-reactivity will differ for each receptor. This could lead to the functions associated with the receptor that has a high degree of cross-reactivity to be overrepresented compared to the function mediated by the poorly cross-reactive receptor.

smooth muscle, chondroblasts, osteoblasts and fibroblasts will produce 'poor relative' mouse IL-6, which can only activate mouse cells but not human cells [5]. As if the situation is not complicated enough, the IL-6 receptor is expressed not only by many leukocytes (T cells, activated B cells, monocytes), but also by nonleukocytes such as hepatocytes, where it plays a role in induction of acute-phase proteins. In some humanized mice (namely BLT mice [4]) human liver cells will be present. These cells could potentially be activated by human IL-6 but not by mouse IL-6, while the mouse liver cells can be activated by both mouse and human IL-6. In other humanized mice models, no human liver cells are present, potentially giving rise to very different outcomes.

Another complex situation arises when a cytokine has the ability to interact with more than one receptor (Fig. 9.2). For example, according to the KEGG cytokine–cytokine receptor interaction pathway (http://www.genome.jp/kegg/pathway/hsa/hsa04060.html), TNF superfamily member 10 (TNFSF10) also known as TNF-related apoptosis inducing ligand (TRAIL) or APO-2 ligand can bind five different receptors (TRAILR1-4 and TR1). In another example, the IL-15 receptor can consist of different subunits, which combine to form different receptors with different affinities for both the human and mouse IL-15 giving rise to complex titration curves, which differ on different cells [2]. Thus, it is possible that the degree of cross-reactivity of the human and mouse cytokine will differ between each of these receptors expressed on different cells and/or associated with different functions. This creates an imbalance between these functions that are mediated by cross-reactive versus

species-specific receptors or receptor complexes in the presence of the cytokine from the nonmatching species (Fig. 9.2).

9.3.2 Multi-Subunit Cytokines

The case of subunit cytokines is also interesting, as species-specificity reactivity does not necessarily needs to apply equally to both subunits giving rise to some interesting possibilities. For example, IL-12 is composed of a p35 and a p40 subunit with the heterodimer being active the active cytokine [11]. While murine IL-12 activates human cells, human IL-12 does not activate mouse cells. Studies using interspecies heterodimers demonstrated that the p40 subunit was cross-reactive and that the p35 subunit was responsible for the observed asymmetrically species specificity [12]. Interestingly, while p35 is expressed in both lymphoid tissue (e.g. spleen and thymus) and nonlymphoid tissue (e.g. lung and brain), the p40 subunit is expressed only in lymphoid cells [12]. In addition, the p40 homodimer is a competitive inhibitor of the heterodimer in that it binds the receptor yet does not activate the cell. Presumably, due to the cross-reactivity of the p40 in the heterodimer, the p40 homodimer will also be cross-reactive between human and mouse and inhibit both cell types. To complicate matters further, the cross-reactive p40 chain can also associate with another polypeptide, p19 to form the cytokine IL-23, whereas the species-specific p35 chain can associate with the Ebi3 chain to form IL-35 [13].

Hence under circumstances when species-specific cytokines are: (i) made by cells of both mouse and human origin, (ii) have receptors that are expressed on cells from both mouse and human origin, (iii) have more than one receptors mediating different functions and/or (iv) composed of multiple subunit; extreme care needs to be taken when interpreting findings in humanized mice.

9.4 Cross-Reactivity and Amino Acid Identity of Humans and Mice Cytokines

Arguments put forward in the previous section suggest that cytokine species specificity is highly relevant to humanized mice. Hence, it is worthwhile investigating what is known about cross-reactivity of cytokines between mice and humans. Obviously, species specificity of cytokines, can best be determined experimentally using bioassays or receptor binding studies. However, this is time consuming and in many cases the results of these experiments, especially when negative, are likely to be underreported. Across a broad range of species the ability of cytokines to cross-react between species is linked to their percentage amino acid identity [14]. In general terms, the higher the amino acid identity between equivalent cytokines in different species, the more the three-dimensional, physical and chemical structures of the proteins will be similar and therefore the higher the probability that cross-reaction

will occur. Therefore, in the absence of experimental data, an informed guess can be made about the likely cross-reactivity, based on amino acid identity between the homologous cytokines in human and mice. This is obviously only a very rough tool and should be considered in terms of statistical probability of cross-reaction rather than as a replacement of experimental determination of cross-reaction.

In Tables 9.1, 9.2 and 9.3, cytokines as defined in the KEGG database (http://www.genome.jp/kegg/brite.html), were ranked by percentage of amino acid identity between human and mouse cytokine homologues. The amino acid identity between mouse and human cytokines varies from 100 to 31%. At above 80% amino acid identity (Table 9.1) there is a high probability of the cytokines being cross-reactive between human and mouse and therefore these cytokines particularly in the higher end of the amino acid identity scale, are expected to be of little consequence in humanized mice. In a range between approximately 80 and 60% amino acid identity between cytokines of different species (Table 9.2), there is a gray zone where cross-reaction does occur but in many cases there is either no cross-reaction or only one-way cross-reaction. Below, 60% amino acid identity (Table 9.3) it is expected that there will be much less chance of cross-reaction occurring, particularly the two-way cross-reaction that is often important in humanized mouse models.

9.5 Interactions Between the Pathogen and the Humanized Mouse

Many pathogens, especially viruses, have evolved to evade immune responses through a large number of strategies. Some of these strategies involve immunomodulation at the level of cytokines including the use of cytokine analogues, cytokine receptor analogues and molecules interfering with cytokine production or its effect on target cells [19, 20]. In many cases viruses have 'stolen' host cytokine-related genes and used these to subvert immune responses [20]. As a result of selection pressure exerted by the immune system on the pathogen these cytokine-related viral genes are critical to the survival of the virus and are important in the context of vaccine development [21]. The capture of cytokine genes by viruses and their subsequent selection can only occur in the species, which are the natural host to the infective agent. Indeed, there is little opportunity, and no selection pressure, for obtaining immunomodulatory mechanisms from species that are not naturally infected by the pathogen, suggesting that immunomodulatory mechanisms might to some degree be species specific. Therefore, even if, under experimental setting, the pathogen can be made to infect the species used as a model, it is unlikely that all immunomodulatory mechanisms would be active in that experimental model. Thus in these cases, the model would not mimic all immunomodulatory function that the pathogen has in its natural host and at least theoretically, important immune evasion mechanisms might be missed.

Applying this insight to humanized mouse models of HIV is not easy for a number of reasons. First, it is not possible to predict as yet undiscovered immunomodulatory

Table 9.1 Cytokines from the KEGG database likely to (at least one-way) cross-react between mice and humans based on percentage of amino acid identity between humans and mice

KEGG	Cytokine	%	KEGG	Cytokine	%
K04358	Fibroblast growth factor 12	100	K12457	Neurotrophin 4	91
K04358	Fibroblast growth factor 8	100	K05460	Hepatocyte growth factor	91
K04358	Fibroblast growth factor 16	100	K13375	Transforming growth factor beta-1	90
K04358	Fibroblast growth factor 9	100	K05459	Insulin-like growth factor 1	90
K04358	Fibroblast growth factor 18	99	K13376	Transforming growth factor beta-2	90
K05497	Growth differentiation factor 11	99	K04359	Platelet derived growth factor B	90
K04358	Fibroblast growth factor 14	99	K05474	TNF ligand superfamily member 12	89
K04358	Fibroblast growth factor 17	99	K05462	Ephrin-A 2	89
K13377	Transforming growth factor beta-3	98	K05457	Neuregulin 3	88
K05465	Angiopoietin 1	97	K05490	Interleukin 17B	88
K04663	Bone morphogenetic protein 7	97	K04358	Fibroblast growth factor 3	88
K04358	Fibroblast growth factor 11	97	K05417	Interleukin 11	88
K04662	Bone morphogenetic protein 4	97	K05448	Vascular endothelial growth factor B	88
K05463	Ephrin-B	97	K05462	Ephrin-A 5	87
K04355	Brain-derived neurotrophic factor	97	K04664	Growth differentiation factor 6	87
K05421	B-cell stimulating factor 3	97	K05450	Platelet derived growth factor C	87
K04667	Inhibin, beta B	96	K05456	Neuregulin 2	86
K05497	Growth differentiation factor 8	96	K04663	Bone morphogenetic protein 8A	86
K04667	Inhibin, beta A	96	K05466	Angiopoietin 2	86
K05463	Ephrin-B 1	96	K04358	Fibroblast growth factor 5	86
K04356	Neurotrophin 3	96	K02582	Nerve growth factor, beta	85
K05463	Ephrin-B 3	96	K05449	Vascular endothelial growth factor C	85
K04358	Fibroblast growth factor 1	96	K05503	Bone morphogenetic protein 10	85
K05502	Bone morphogenetic protein 1	95	K05492	Interleukin 17D	85
K04358	Fibroblast growth factor 20	95	K05450	Platelet derived growth factor D	85

Table 9.1 (continued)

KEGG	Cytokine	%	KEGG	Cytokine	%
K10033	C-X-C motif chemokine 14	95	K05473	TNF ligand super-family memb. 11	85
K04358	Fibroblast growth factor 2	95	K13769	Insulin-like growth factor 2	84
K05480	Ectodysplasin-A	95	K05449	Vascular endothelial gr. factor C	84
K04358	Fibroblast growth factor 7	94	K05462	Ephrin-A 1	84
K05455	Neuregulin 1	94	K05420	Ciliary neurotrophic factor	83
K04358	Fibroblast growth factor 13	94	K05424	Leptin	83
K10031	C-X-C motif chemokine 12	93	K05496	Bone morphogenetic protein 3B	83
K04358	Fibroblast growth factor 10	93	K05461	KIT ligand[a]	83
K04358	Fibroblast growth factor 6	93	K05462	Ephrin-A 4	83
K05462	Ephrin-A 3	93	K04667	Inhibin, beta E	82
K04663	Bone morphogenetic protein 5	93	K05488	IL-1 family, member 10 (theta)	82
K05452	Glial cell-derived neuro-trophic factor	93	K04668	Left–right determi-nation 2	82
K04359	Platelet derived growth factor A	92	K04668	Left–right determi-nation 1	82
K04662	Bone morphogenetic protein 2	92	K05464	Growth arrest-specific 6	82
K04664	Growth differentiation factor 5	91	K04358	Fibroblast growth factor 22	81
K04663	Bone morphogenetic protein 6	91	K05453	Macrophage CSF 1[b]	81
K05483	IL-1 family, member 5 (delta)	91			

[a] Mouse active on human cells, but human not active on mouse cells (SCF [5])
[b] Human active on mouse cells, but mouse not active on human cells [5]

mechanisms. As such, it is not possible to experimentally determine whether these would affect the humanized mice. Fortunately, most of the known immunomodulatory mechanisms affect cells directly involved in immune regulation [22]. In the case of humanized mice these would be predominantly transferred human cells. Since these cells are derived from the natural host, there should be no problem. However, it is impossible to exclude that some immunomodulatory mechanisms would be initiated or would contain at least one step that involves a nonleukocyte and therefore a mouse cell. For example it is well documented that HIV has the ability to interfere with type I IFNs [23]. In a humanized mouse sources of type I IFNs would include, human leukocytes (B cells, T cells, NK cells, macrophages and dendritic cells) as well as murine endothelial, epithelial cells and some neurons. Since

Table 9.2 Cytokines from the KEGG database that might cross-react (possibly asymmetrically) between mice and humans based on percentage amino acid identity between human and mouse

KEGG	Cytokine	%	KEGG	Cytokine	%
K05448	Vascular endothelial growth factor A	80	K05444	Interleukin 19	70
K12499	C-C motif chemokine 5	80	K05495	Growth differentiation factor 1	70
K05503	Bone morphogenetic protein 9	80	K05407	Platelet factor 4	70
K04358	Fibroblast growth factor 4	80	K05454	Fms-related tyrosine kinase 3 ligand	69
K05496	Bone morphogenetic protein 3	79	K12671	C-X-C motif chemokine 10	68
K05500	Inhibin, alpha	79	K05505	C-X-C motif chemokine 1	68
K04358	Fibroblast growth factor 21	79	K05416	C-X-C motif chemokine 9	68
K03156	TNF superfamily, member 2[a]	79	K12672	C-X-C motif chemokine 11	68
K04666	Nodal	79	K14624	C-C motif chemokine 2	68
K05422	Cardiotrophin 1	79	K04519	Interleukin 1 beta	68
K05437	Erythropoietin	79	K05425	Interleukin 12B[b]	67
K04664	Growth differentiation factor 7	78	K05438	Growth hormone 1	67
K05445	Interleukin 22	78	K05447	Interleukin 28A	67
K05491	Interleukin 17C	78	K05448	Vascular endothelial gr. factor A/B	66
K05512	C-C motif chemokine 19	78	K05444	Interleukin 24	66
K03161	TNF ligand superfamily member 5	77	K05482	Interleukin 18	66
K05477	TNF ligand superfamily member 14	77	K05513	C-C motif chemokine 28	66
K05493	Interleukin 17E	77	K05514	C-C motif chemokine 17	64
K05481	Interleukin 1 receptor antagonist	76	K05508	C-X3-C motif chemokine 1	64
K12964	C-C motif chemokine 4	76	K05414	Interferon alpha 13	64
K05419	Leukemia inhibitory factor	76	K05414	Interferon alpha 8	64
K05478	TNF ligand superfamily member 15	76	K14625	C-C motif chemokine 20	64
K04667	Inhibin, beta C	76	K04357	Epidermal growth factor	64
K05444	Interleukin 20	76	K05447	Interleukin 28B	63
K05458	Neuregulin 4	76	K05498	Growth differentiation factor 9B	63

Table 9.2 (continued)

KEGG	Cytokine	%	KEGG	Cytokine	%
K05408	C-C motif chemokine 3	75	K05511	C-C motif chemokine 15/23	63
K05426	Interleukin 23, alpha subunit p19	75	K05414	Interferon alpha 1	63
K00431	Thyroid peroxidase	74	K05514	C-C motif chemokine 22	63
K04663	Bone morphogenetic protein 8B	74	K05505	C-X-C motif chemokine 2	63
K04389	TNF ligand superfamily member 6	74	K05486	Interleukin 1 family, member 8 (eta)	63
K03157	Lymphotoxin beta	73	K05513	C-C motif chemokine 27	63
K05475	TNF ligand superfamily member 13	73	K05509	C-C motif chemokine 7	62
K04665	anti-Mullerian hormone	73	K05489	Interleukin 17	62
K05443	Interleukin 10	73	K05504	Growth differentiation factor 15	62
K05433	Interleukin 15[b,c]	73	K05431	Interleukin 7[b]	62
K05468	Lymphotoxin alpha	72	K05510	C-C motif chemokine 6/9	61
K05498	Growth differentiation factor 9	72	K04383	Interleukin 1 alpha	61
K05512	C-C motif chemokine 21C	71	K05439	Prolactin	61
K05423	Granulocyte CSF[b]	71	K05414	Interferon alpha 6	61
K05495	Growth differentiation factor 3	71	K05434	Interleukin 21	61
K05512	C-C motif chemokine 21A	71	K05507	C motif chemokine 1	61
K05428	Interleukin 5	71	K05487	Interleukin 1 family, member 9	60
K04358	Fibroblast growth factor	71	K05414	Interferon alpha 5	60
K05471	TNF ligand superfamily member 8	70	K05414	Interferon alpha 7	60

[a] Cross-reaction human cytokine on mouse cells [10]
[b] Cross-reaction between mouse and human [5, 11]
[c] Eisenman et al. [2] suggests large quantitative differences for IL-15

there is no evolutionary pressure for HIV to develop a mechanism to affect mouse cells, the ability of the virus to affect these cells would be fortuitous. In addition, little data is available about the species specificity of type I IFNs (many different proteins all with the potential to show at least some species specificity), further complicating interpretation of results. Hence, studies involving immunomodulatory mechanisms, particularly if they contain at least one interaction with mouse-derived cells, could be problematic.

Table 9.3 Cytokines from the KEGG database unlikely to be cross-react both ways between mice and humans based on percentage amino acid identity between human and mouse

KEGG	Cytokine	%	KEGG	Cytokine	%
K13072	C-C motif chemokine 25	59	K05479	TNF ligand superfamily member 18	52
K05442	Interferon, tau-1	59	K05506	C-X-C motif chemokine 5	51
K05414	Interferon alpha 2	59	K04358	Fibroblast growth factor 15/19	51
K05514	C-C motif chemokine 24	59	K10029	Platelet basic protein	50
K05509	C-C motif chemokine 11	59	K05514	C-C motif chemokine 26	50
K05406	Interleukin 12A[a]	59	K05415	Interferon beta	49
K05435	Interleukin 13	59	K05418	Oncostatin M	49
K04721	TNF ligand superfamily member 10	58	K10035	C-X-C motif chemokine 16	48
K05414	Interferon alpha 4	58	K10032	C-X-C motif chemokine 13	47
K05429	Interleukin 2[b]	58	K05469	TNF ligand superfamily member 4	45
K05414	Interferon alpha 14	57	K05514	C-C motif chemokine 1	42
K05494	Interleukin 17F	57	K05436	Thymic stromal lymphopoietin	42
K05432	Interleukin 9	57	K05430	Interleukin 4[c]	41
K05470	TNF ligand superfamily member 7	56	K04687	Interferon gamma[c]	41
K05476	TNF ligand superfamily member 13B	56	K05405	Interleukin 6[b]	41
K05427	Granulocyte-macrophage CSF[c]	55	K05441	Interferon, kappa	37
K05467	Angiopoietin 4	55	K05472	TNF ligand superfamily member 9	34
K05484	IL-1 family, member 6 (epsilon)	55	K04736	Interleukin 3[c]	31
K05509	C-C motif chemokine 8	54			

[a] Mouse cytokine active on human cells, but human not active on mouse cells [11]
[b] Human cytokine active on mouse cells, but mouse not/less active on human cells [2, 5]
[c] No cross-reaction [5, 9, 15–18]

9.6 Concluding Remarks

When considering animal models involving combining cells from mice and humans it is critical to consider that a proportion of the cytokines will not cross-react between species and that this lack of cross-reaction might well be asymmetrical. This has important implications not only for the development of the transplanted human

immune cells but also for the induction of immune responses. In particular, complications can arise at the interface between the transplanted immune system and the host, including, for example, the interactions between the nervous system and the immune system. While some of these issues can be specifically addressed by creating new models in which certain human cytokines are knocked-in and therefore address specific concerns, only a very limited number of the many non-cross-reacting cytokines can be manipulated in that way. As a result when considering animal models it is imperative to focus on certain aspects of the host/pathogen interaction in which these issues are not critical, while acknowledging that the model is unsuitable for the study of other aspects. Ignoring these issues might result in some findings not being able to be translated to the situation in humans hence potentially misleading researchers in their quest to disease understanding and control.

Finally, the species specificity issues highlighted here for cytokines are equally valid for a range of other proteins that are produced by transplanted cells from humans and affect or are affected by proteins from the mouse host. These could include but are not limited to, proteins involved for example in the formation of immune organs or expressed on mouse endothelial cells and involved in the regulation of immune cell trafficking.

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