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Klinický význam polymorfismu cytokinových genů

Clinical significance of cytokine gene polymorphism

Disertační práce

Vedoucí závěrečné práce/Školitel: Prof. MUDr. Ilja Stříž, CSc

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Prohlášení:

Prohlašuji, že jsem závěrečnou práci zpracoval samostatně a že jsem uvedl všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, 21. 5. 2012

Podpis

Abstrakt

Lidský genom je plný různých sekvenčních variant. Liší se hlavně velikostí ale také svým vlivem na fenotyp. Nejmenší jednotkou genetického polymorfismu je tzv. jednonukleotidový polymorfismus (SNP z angl. single nucleotide polymorphism). SNP jsou tvořeny záměnami jednotlivých bazí mezi dvěmi alelami a mohou ovlivnit genovou expresi. Genetické polymorfismy jsme studovali ve třech oblastech: (1) jako marker rizikových pacientů po orgánové transplantaci, (2) diagnostický marker u pacientů s interticiálními plicními chorobami nebo (3) s myomy. Dospěli jsme k následujícím závěrům.

Etnikum nebo národnost hrají svou roli v distribuci genetických polymorfismů. Toto musíme brát do úvahy, když se snažíme porovnat s naší populací výsledky z jiných populací. Naše první klinická studie s asociací genů dospěla k nálezu, že dokonce genový polymorfismus cytokinu IL-18 může přispět k opoždění nástupu funkce štěpu po transplantaci ledviny a podporuje roli tohoto prozánětlivého cytokinu v časné imunitní odpovědi proti ledvinnému aloštěpu. Při studiu intersticiálních plicních chorob (IPCH) jsme dospěli k závěru, že genový polymorfismus cytokinů přispívá k patogenezi a hraje roli v etiologii IPCH s důrazem na promotorové oblasti v IL-4, IL-4RA, IL-1RA a IL-12. Našli jsme asociace mezi polymorfismy a funkčními parametry idiopatické plicní fibrózy (IPF) a mezi hladinami ostatních cytokinů. A konečně, jak se cytokiny podílejí na rozvoji děložního myomu, tak se zdá, že jejich genové polymorfismy rovněž hrají určitou roli, což jsme demonstrovali na polymorfismu v genech pro IL-4 a tumor nekrotizující faktor (TNF)-α.

Naše data podporují hypotézu, že genové polymorfismy mohou ovlivnit imunitní reakce. Ovšem míra tohoto ovlivnění není dosud známa. Procesy při orgánové transplantaci nebo patogeneze choroby jsou ovlivněny mnoha geny a jsou tak komplexní, že si těžko dovedeme představit, že by jediná bodová záměna v konkrétním genu mohla ovlivnit tolik biochemických a signalizačních drah. Musíme vzít také do úvahy roli epigenetiky, která se zdá, že hraje určitou významnou roli.

Abstract

The human genome is full of different sequence variants. They are different mainly in size but also in their influence on phenotype. The smallest unit of genetic polymorphism is single nucleotide polymorphism (SNP). SNPs represent a single nucleotide change between two alleles and might affect the gene expression. We have studied SNPs in three distinct fields as: (1) marker of risky patients after the organ transplantation, (2) diagnostic marker of patients with interstitial lung diseases (ILD) or (3) with uterine fibroid (UF). We have come to the following results.

Ethnicity or even nationality plays a role in the distribution of genetic polymorphism. This must be absolutely taken into account when one would like to transfer findings of a clinical study from a certain nation or ethnic and applied them to his studied group for the comparative purposes. Our first clinical gene-association study has found that even gene polymorphism of the IL-18 gene may contribute to the delayed onset of kidney graft function after transplantation and support the role of this proinflammatory cytokine in the modulation of early immune responses against the kidney allograft. From the studies of ILDs, we conclude that cytokine gene polymorphisms contribute to the pathogenesis and play a role in the etiology of ILDs, with an emphasis on the IL-4, IL-4RA, IL-1RA, and IL-12 promoter regions. Associations have been found between the polymorphisms and functional parameters of idiopathic pulmonary fibrosis (IPF) and levels of other cytokines. Finally, as cytokines play a role in the development of UF it seems that their gene polymorphisms play a role as well as we demonstrated on the polymorphism for IL-4 and tumor necrosis factor (TNF)-α.

Our data suggest that the gene polymorphism can influence immune reaction. However, the strength of this effect is still unknown. Processes in organ transplantation or in disease pathogenesis are so polygenic and complex that it is hard to imagine that one point substitution in the certain gene could affect so many biochemical and signalling pathways. We have to take into account also a role of epigenetics on the level of gene expression which also seems to play a significant role.

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Chapters

List of abbreviations

Abbreviation	Description
ACE	Angiotensin converting enzyme
AMY1	Gene encoding salivary amylase
APC	Antigen presenting cell
ATS	American Thoracic Society
BAL(F)	Bronchoalveolar lavage (fluid)
bFGF	Basic fibroblast growth factor
BLM	Bleomycin
CCR5	CC chemokine receptor 5
CF	Cystic fibrosis
CGP	Cytokine gene polymorphism
CMV	Cytomegalovirus
CNP	Copy number polymorphism
CNV	Copy number variations
COPD	Chronic obstructive pulmonary disease
CXCR3	CXC chemokine recetor 3
DGF	Delayed graft function
DL(CO)	Diffusing capacity for carbon monoxide
EAA	Extrinsic allergic alveolitis
EBV	Epstein-Barr Virus
EC	Endothelial cell
ECM	Extracellular cell matrix
EGF	Epidermal growth factor
ENA-78 (CXCL5)	Epithelial neutrophil-activating protein 78
ERS	European Respiratory Society
FGF	Fibroblast growth factor
G-CSF	Granulocyte colony stimulating factor
GM-CSF	Granulocyte-macrophage colony stimulating factor
GnRH	Gonadotropin releasing hormone
HHV-6	Human herpesvirus 6
HLA	Human Leukocyte Antigen
HP	Hypersensitivity pneumonitis
HRCT	High resolution computed tomography
HSV	Herpes simplex virus
ICAM-1	Intercellular adhesion molecule-1
IFN-γ	Interferon- γ
IL-	Interleukin-
IL-8 (CXCL8)	Interleukin-8
ILD	Interstitial lung diseases
IP-10	Interferon-inducible protein 10
IPCH	Intersticiální plicní choroby
IPF	Idiopathic pulmonary fibrosis
I-TAC (CXCL11)	Interferon-inducible T-cell alpha chemoattractant
LCV	Large-scale CNVs

Abbreviation	Description
LFA-1	Lymphocyte function-associated antigen-1
MAF	Macrophage activating factor
MCF	Macrophage chemotactic factor
MCP-1 (CCL2)	Macrophage chemotactic protein 1
MDC (CCL22)	Macrophage-derived chemokine
MHC	Major histocompatibility complex
MIF	Macrophage inhibition factor
MIG (CXCL9)	Monokine induced by gamma-interferon
MIP-1α (CCL3) MIP-1β (CCL4)	Macrophage inflammatory protein 1-alpha/beta
MMP	Matrix metalloproteinase
mRNA	Messenger RNA
NK	Natural killer
OR	Odds ratio
PDGF	Platelet derived growth factor
PDGFR	Receptor for platelet derived growth factor
RA	Rheumatoid arthritis
RANTES	Regulated upon Activation Normal T-cell Expressed and presumably Secreted
RF	Risk factor
SNP	Single nucleotide polymorphism
TARC (CCL17)	Thymus and activation regulated chemokine
TCR	T-cell receptor
TGF-β	Transforming growth factor beta
Th	Helper T lymphocyte
TNFA	Gene for tumor necrosis factor alpha
TNFR1	TNF-receptor 1
TNF-α	Tumor necrosis factor alpha
Tpo	Thymopoietin
UF	Uterine Fibroid
UTR	Untranslated region
VC	Vital capacity
VCAM-1	Vascular cellular adhesion molecule 1
VEGF	Vascular Endothelial Growth Factor

Gene polymorphisms

We can distinguish several chromosome variants in the human genome. They are different mainly in size but also in their influence on phenotype.

The smallest unit of genetic polymorphism is ***single nucleotide polymorphism (SNP)***. SNPs represent a single nucleotide changes between two alleles found in more than 1% of chromosomes in a given population. It can occur in both coding and noncoding parts of the gene and thus they might or might not be reflected in the protein product. It is estimated that approximately 10 million SNPs had been identified across the human population (Kruglyak et al. 2001; Lander et al. 2001; "Finishing the euchromatic sequence of the human genome" 2004). We can find the most well known SNP in the human ABO blood groups, Rh factor or major histocompatibility complex (MHC). The consequence of such genetic variation is, for instance, largely variability of the human MHC genes (> 6 000 alleles) or the existence of a defective enzyme or changes in the gene expression. HapMap, the biggest international project at presents, is focused on mapping SNPs throughout the world populations to identify all possible SNPs. Their results are necessary for future studies aimed at SNP associations with diseases.

Microsatellites (e.g. CA_n repeats) are described as sequences with 1-6 repeats totalling <200 bp in length. The frequency is estimated as more than 1 million (~3 %) of the human genome. A larger kind of these repeats are called ***Minisatellites***. They are formed by 20 – 50 copies of 6-100 bp. Their frequency lies around 150 000 minisatellites.

"InDels" – insertions/deletions belong among other well known gene variants. These types of variations cover a segment of DNA causing small polymorphic changes but also large chromosomal aberrations. InDels more than 1 kb in size are often also called ***Copy Number Vartions (CNVs)***. If the frequency is more than 1 %, these are called ***Copy Number Polymorphism (CNP)***. We can also distinguish ***large-scale CNVs (LCV)*** covering approximately 50 kb in size or greater. More CNVs

usually means more gene product and, consequently, more protein is encoded. Such an example is represented by gene AMY1 (encoding salivary amylase). Populations whose diet is rich in starches (e.g. Americans or Japanese) posses 7 copies on average, while populations whose diet is rather rich on dairy products and fish have only 5 copies on average.

If we go larger in size, we can further distinguish **Inversions** and **Translocations** where whole segments of the chromosomes are cut and paste in reverse direction or rearranged from one chromosome to another. However, the scope of this dissertation is focused primarly on cytokines and their single nucleotide polymorphisms.

How are polymorphisms useful?

Analysis of polymorphisms can be used for:

- Tissue typing.
- Finding disease genes.
- Population studies, e.g. assessing the degree of genetic diversity in a population.
- Determining whether two populations represent separate species or races of the same species.
- Tracking migration patterns of a species.

The role of cytokines in organ transplantation

Organ transplantation has become a standard therapeutic procedure in treating the end stage diseases of the kidneys, liver, heart or lungs. The main problem of organ transplantation is an immune reaction developed against host or donor cells leading to the loss of graft function or, in problematic cases, even to graft rejection. Present immunosupressive protocols can assure about 90 % patient survival after the first year of transplantation and 70-90 % graft survival differing among transplant centres. However, long-term usage of immunosupressive drugs leads to complica-

tions like opportune infections, cardio-vascular diseases or progression of secundar malignancy. Understanding the mechanisms of graft rejection or graft adaption could help us to target immunosupressive drugs and to lower its dosage.

However, searching for the graft adaption is still at present elusive. Currently, we know quite a lot about the role of human leucocyte antigens (HLA) and matching in recognition of graft rejection but we have also realised that there are many other factors contributing to a development of rejection such as: ischemia/reperfusion damage, surgical complications, infections, hypertension, hyperlidimeia, nefrotoxicity of immunosuppression, donor age or sex or brain death. Beside all these factors and HLA matching, there are other variables like polymorphisms in minor antigens or cytokine genes playing a prominent role in the mechanisms of graft rejection.

Immunobiology of graft rejection

The first rejection reaction that can occur after transplantation is the ***acute rejection.*** This process begins with the presentation of donor alloantigens to recipient T cells through antigen presenting cells (APC) or by a direct mechanism. The central role belongs to T cells and their T-cell receptor (TCR) distinguishing HLA alloantigens. This specific immune response occurs in two main forms (Figure 1). In the first pathway, called ***direct allorecognition***, donor antigens are presented by donor APC to recipient T lymphocytes, which further became activated, proliferate and differentiate. APC are represented by dendritic cells that are transported together with the transplanted organ and invade the host lymphoid organs where they stimulate host T cells. Such activated T cells are then recruited to the transplanted organ. T cells further create such a cytokine milieu which stimulates growth and leads to differentiation of other cells. For example, interferon-γ (IFN- γ) can stimulate renal tubular cells, endothelial cells, and pancreatic beta cells to express HLA class II molecules (Paul 1989). In the second pathway, ***indirect allorecognition***, donor graft-derived antigens (HLA and non-HLA) are presented to recipient T lymphocytes by recipient APC (Butcher et al. 1982; Rock et al. 1983; Golding et al. 1984; Sherwood et al. 1986; Fangmann et al. 1992; Liu et al. 1992). While the direct/indirect antigen presentation is central to acute rejection, an indirect pathway of allorecognition provides continuing antigenic stimulus for ***chronic allograft rejection*** (Kappler et al.

1987; Cramer et al. 1989; Auchincloss et al. 1996; Shirwan 1999). This is a long-term process leading to organ damage. Cells infiltrating an organ are macrophages, T cells (mainly CD4+), plasma and natural killer (NK) cells. Mechanisms of chronic rejection arise from the cooperation of immunological and non-immunological factors. The consequence of this collaboration is damage of endothelial cells (EC) and their activation. Activated endothelial cells express more adhesive molecules (Intercellular adhesion molecule (ICAM)-1, Vascular cellular adhesion molecule (VCAM)-1, P and E-selectins) regulating the infiltration of immunocompetent cells (Sis et al. 2010). EC are also a source of chemokines (IL-8, Macrophage chemotactic protein (MCP)-1, Macrophage inflammatory protein (MIP)-1α, MIP-1β, Regulated upon Activation Normal T-cell Expressed and presumably Secreted (RANTES)) attracting other leukocytes to the organ. Furthermore, activated EC express HLA antigens class I and II thus they are capable of T-cell stimulation (Briscoe et al. 2002; Kreisel et al. 2002). Beside these features, EC can also produce cytokines like TNF-α or platelet derived growth factor (PDGF).

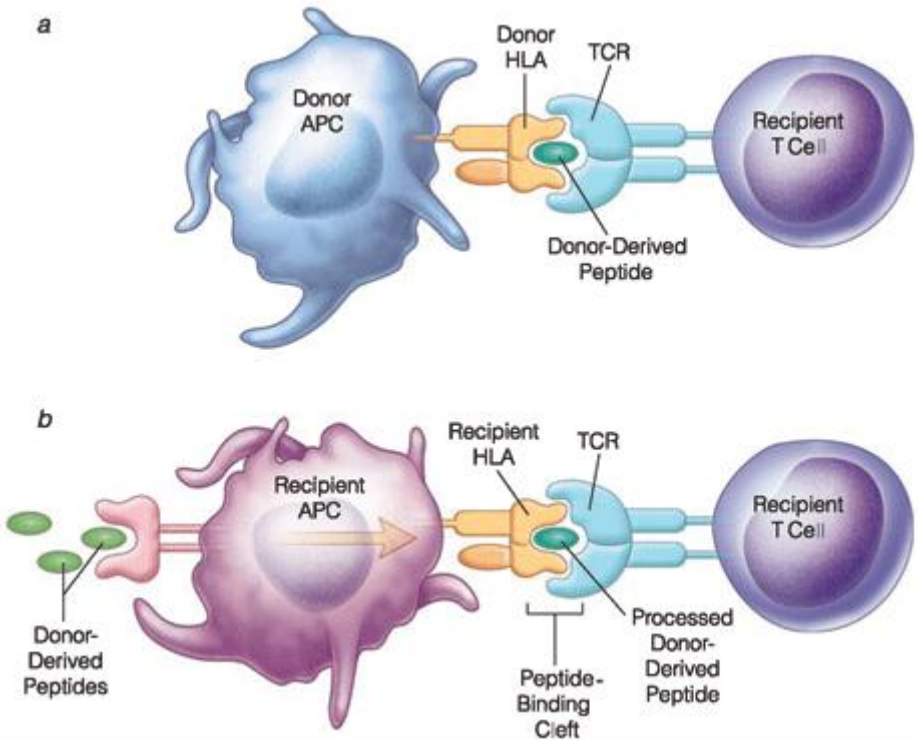


Figure 1 **(*a*) Direct allorecognition.** T cells responding to direct antigen presentation by donor-derived antigen-presenting cells (APCs) recognise determinants on the allogeneic HLA molecule as well as structures found on

the bound peptide. **(*b*) Indirect allorecognition.** Indirect recognition requires antigen processing and presentation by recipient-derived APCs. HLA = human leukocyte antigen; TCR = T cell receptor. (adopted from Section 10/Chapter 12 Transplant Immunology: Basic Immunology and Clinical Practice)

The nature of APCs and their interaction with T-cells lead to either humoral or cell-mediated immune response which is determined by the cytokines produced by the APCs. Cell-mediated immunity is driven by T-helper (Th)1 cells and related cytokines whereas humoral immunity is driven by T-helper (Th)2 cells and their related cytokines. One of the important cytokines to be released is IL-2. Further, IL-3 is synthesised to stimulate stem cell proliferation which in turn induces differentiation of granulocytes and macrophages. IFN-γ is a characteristic cytokine of Th1 cells and it enhances HLA expression on macrophages and potentiates cytotoxicity of T lymphocytes. Th2 cells produce cytokines like IL-4, -5, -6 and -13 which are critical for induction of humoral immunity, isotypic immunoglobulin switching, and eosinophilia.

Cytokine gene polymorphism in organ transplantation

We still cannot achieve a successful long term graft survival despite the considerable progress in immunosuppressive treatment after kidney transplantation, new techniques of organ preservation and reduction of rejection incidence. Factors mentioned above or their combinations are responsible for this insufficient outcome. Due to the lack of organs, marginal donors often have to be chosen. Marginal donors are such individuals who do not fulfill optimal criteria for organ donation, e.g. age plays an important role. Currently, approximately 50 % of all deceased donors are older than 55. Age, ethnicity, HLA compatibility, titre of panel reactive antibodies are the criteria for assessing the immunological status of a patient in the majority of transplant protocols. Despite the evident success of these procedures, the major part of a genetic variability of a transplanted recipient is being ignored which might lead to over or underdosing with immunosuppressives. Over the last 10 years, substantial attention has been given to genetic markers associated or directly contributing to the transplant outcome. We suppose that with this knowledge we could select patients more susceptible, for example, for ischemic-reperfusion damage and thus minimise the risk of delayed graft function.

Cytokines and chemokines form a family of secreted proteins intermediating and regulating immune response, inflammation and haemopoiesis. Logically, a lot of scientists have focused mainly on the gene variants of these genes. It has been shown in different studies, that SNPs or repetitive sequences in the promoter, coding and non-coding, 5´UTR or 3´UTR (untranslated region) sequences can up/down regulate the gene expression.

Cytokines

We review here genetic variants of the following cytokines, because they were repeatedly observed in different studies of different cohorts either with positive or negative results.

TNF-α is one of the key cytokines stimulating and regulating inflammatory responses. It affects APCs and stimulates them for antigen processing, potentiates HLA class II expression and thus cooperates in APC maturation. There is a variable position -308 in its promoter region, in which the G⇒A substitution is associated with 6 to 7 fold higher transcription and protein expression in vitro (Wilson et al. 1997). The functional result of this SNP seems to be more than convincing; however the situation is more complicated when comparing different association studies concerning this SNP with transplantation outcome. Despite several studies which have proved a relation between SNP -308 A/G and the graft rejection, there is an important multicentric study performed on a cohort of 1901 kidney retransplanted patients which proved the negative effect of TNF-α -308 A allele on the 3-year graft survival (Wilson et al. 1997; Sankaran et al. 1999; Pelletier et al. 2000; Poli et al. 2000; Hahn et al. 2001; Fernandes et al. 2002; Alakulppi et al. 2004; Mytilineos et al. 2004). This is confirmed by other studies failing to confirm positive findings (Marshall et al. 2000; Hutchings et al. 2002; Muller-Steinhardt et al. 2004; Wramner et al. 2004; Dmitrienko et al. 2005).

Within the promoter of IL-10 gene there are three SNPs increasing the cytokine production in stimulated lymphocytes: –1082G, -819C and –592C alleles (Turner et al. 1997). We could suppose that upregulation of anti-inflammatory IL-10 will have a protective effect on the transplantation; however, several studies disprove this hypothesis and have observed an increased risk of acute rejection (Sankaran et

al. 1999; Pelletier et al. 2000; Hutchings et al. 2002). This observation is in concordance with the results of the large multicentric study dealing with IL-10, transforming growth factor (TGF)-β and kidney transplantation with a cohort of 1087 patients. They found an association of the haplotype ACC of IL-10 promoter (–1082, -819 and –592) which predisposing individuals to higher gene production with a worse transplantation outcome (Thakkinstian et al. 2008).

There are also studies combining the potential influence of both cytokines together on acute renal graft rejection. Sankaran with his coworkers (Sankaran et al. 1999) have shown that patients with genetically higher cytokine expression (TNF-α -308 G and IL-10 -1082 G) also have higher risk of acute rejection if the patient and his/her donor is HLA-DR mismatched, whilst patients with only TNF-α -308 G allele have a higher number of acute rejection episodes resistant to steroid treatment.

IL-6 is produced by different cell types involved in the innate or specific immune response. Three SNPs were identified in its promoter region: -597 G/C, -572 G/A and –174 G/C. Functional analysis revealed a cooperative effect of these SNPs on the transcriptional activity (Terry et al. 2000). However, up to now the effect of individual polymorphisms on the incidence of kidney graft rejection was not clearly proved. On the other hand, it has been found that higher IL-6 production (-174 G) positively influences long term kidney graft survival (Marshall et al. 2000; Muller-Steinhardt et al. 2002; Muller-Steinhardt et al. 2004). The effect of -174 G allele was also observed in lower frequency of posttransplant coronary vasculopathy after 5 years from heart transplantation (Densem et al. 2005).

IL-18 (IL1F4) is a member of the IL-1 family. It belongs to proinflammatory cytokines which, on the other hand, is capable of influencing the Th1/Th2 imbalance in Th2 direction in the suitable cytokine milieu. Giedraitis et al. identified 5 SNPs: -656 G/T, -607 A/C, -137 C/G, +113 T/G and +127 C/T. However, only -607 A/C and -137 C/G were confirmed to affect gene expression (Giedraitis et al. 2001). Transcription activity is enhanced by -607 C and -137 G allele. An increased level of IL-18 in sera has been shown to correspond to a higher frequency of acute rejection after kidney transplantation. In addition, patients at higher risk were homozygous for IL-

18 -137 GG. Moreover, GG homozygotes had increased serum level of IL-18 (Kim et al. 2008).

Chemokines

Chemokines form a large family of structurally related chemotactic cytokines participating in cell migration, leukocyte activation, haemopoiesis and angiogenesis and also create a bridge between the innate and specific immunity.

The upregulation of ligands for CC chemokine receptor (CCR) 5 is observed during the acute and chronic kidney rejection and the graft is used to be infiltrated with CCR5+ mononuclear cells. Thirty-two base pair deletion (CCR5Delta32) leads to an inactive receptor. The patients homozygous for this deletion had a significantly longer graft survival in comparison to the other genotypes (Fischereder et al. 2001). This result suggests a possible role of CCR5 in transplant loss and could be of interest as a therapeutic target. The incidence of acute rejection was not influenced. Krüger and his coworkers came to a similar observation. They were interested in SNP -2518 A/G of MCP-1 gene. The G allele upregulates its expression in vitro and carriers of this allele have a 2.5 fold higher level of MCP-1. They did not find an association between SNP -2518 and acute rejection but, more interestingly, they observed that patients with lower MCP-1 expression (-2518 A allele) had better long term graft survival (Kruger et al. 2002). Testing this hypothesis in the group of liver transplant patients failed which might suggest a possible organ specific effect (Schroppel et al. 2002). Two functional polymorphisms were located in the promoter sequence of RANTES gene (CCL5), -403 G/A and -28 C/G, and one in the intron 1 (In 1.1 C/T). The following alleles are associated with upregulation of RANTES gene: -403 A, -28 G and In 1.1 T. Association of -403 GA/AA and In1.1 TC/TT with higher frequency of recurrent acute rejections has been found in patients after kidney transplantation (Kruger et al. 2007).

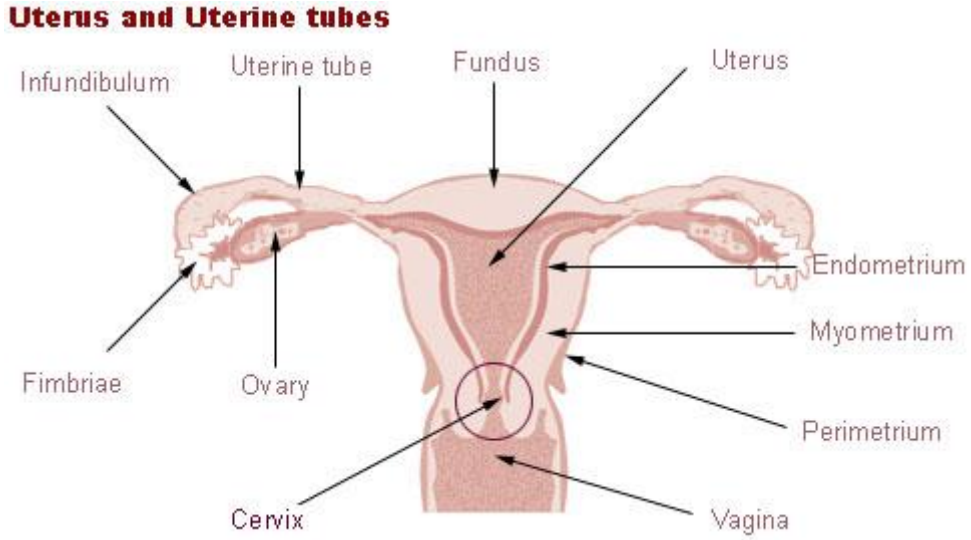
Uterine Fibroid

Characteristics, classification and causes

Uterine fibroid (UF, also known as uterine leiomyoma, myoma, fibromyoma, leiofibromyoma, fibroleiomyoma or fibroma) represents the most frequent benign tumor in women of middle and postmenopausal age that originates from the smooth muscle layer (myometrium) and the accompanying connective tissue of the uterus (Figure 2).

Its prevalence ranges from 20 % to 40 %, but only approximately 1/3 of patients progress to a symptomatic stage (Wallach et al. 2004). A growing tumor causes the most common symptoms like heavy and painful menstruation, painful sexual intercourse, urinary frequency and urgency, infertility and repeated abortions (Haney 2000). UF is often an indication for performing hysterectomy. In very rare cases, UF can develop into a malignant leiomyosarcoma.

Despite its high prevalence, but probably due to its low potential to malignant transformation, the topic of UF is not a focus of interest. In recent years there has been a growing number of studies concerning epidemiology, genetics, hormonal aspects and molecular biology, however, the cause of UF is still not clearly elucidated.



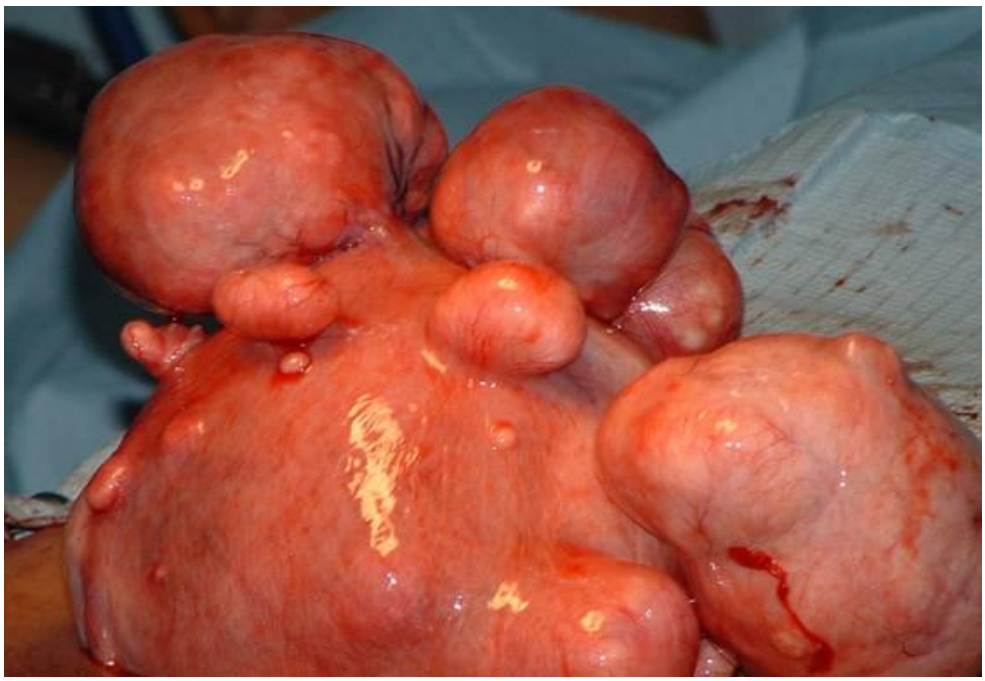


Figure 2 Uterus with multiple uterine fibroids.

Classification

UF may be single or multiple. They usually occur in the intramural location (within the wall of the uterus), thus the most common type is called "**intramural fibroid**" (Figure 3). In the outer layer (serosa or perimetrium) we can distinguish the so called "**subserosal fibroid**". It can grow out in a papillary manner to become "**pedunculated fibroid**". In the inner layer (endometrium) fibroids are classified as "**submucosal**" and they distort the uterine cavity. The pedunculated form is termed "**intracavitary fibroid**". The last form of fibroids is "**cervical fibroid**" located in the wall of the cervix.

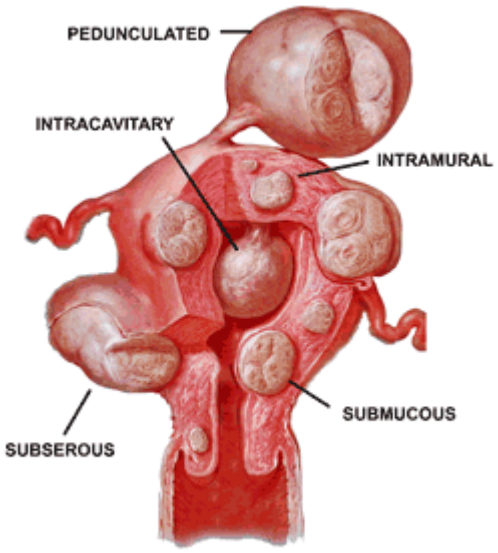


Figure 3: Different types of uterine fibroids.

Causes

Multifactorial model of UF development is supposed and the interaction of different risk factors (RF) is expected. Here we summarise several known risk factors:

- Early onset of menstruation periods (menorrhea) – supposed mechanism is in increased mutagenesis of the cell-cycle controlling genes in the cells of myometrium (Flake et al. 2003).

- Null parity or infertility – it has been shown that multiparous women have decreased incidence of UF (Marshall et al. 1998).
- Older age – age around 40 years is considered as a critical borderline for the UF development (Velebil et al. 1995).
- Body mass index – lowered production of sex hormone binding globulin in the liver of obese women results in the higher levels of free active estrogen in the peripheral blood (Sato et al. 1998).
- Race – the incidence among black women is 2 times higher than in white women (Al-Hendy et al. 2006).
- Protective effect of smoking – nicotine inhibits aromatase that results in the decreased conversion of androgens to estrogens. Smokers also have higher level of sex hormone binding globuline and thus lower level of active free estrogen. Protective effect vanishes in ex-smokers (Schwartz 2001).
- Oral contraceptives – longer ingestion decreases the risk (Schwartz et al. 2000).

Hormones, cytokines and their role

Beside these risk factors, a very important role belongs to hormones and cytokines. It has been recognised a long time ago that estrogen stimulates fibroids. Most recently, there are studies revealing the possible role of progesterone and progestins to fibroid growth as well (Nisolle et al. 1999; Rein 2000). In addition, treatment with antiprogesterone (RU 486) leads to regression of UF and the expression of progesterone receptor in myoma cells also decreases. Treatment with gonadotropin-releasing hormone analogues (GnRH) leads to hypoestrogenic state resulting in the UF regression as well. Cessation of the treatment leads to a rapid renewal of the UF growth almost to its original size (Stovall et al. 1991).

Some authors have demonstrated an increased aromatase activity in UF and thus acceleration of a conversion of estradiol to estron in comparison to normal my-

ometrium (Sumitani et al. 2000). An increased expression of receptors for estrogen and progesterone correlates with these observations.

Mitogenic effect of estrogens is mediated through growth factors produced locally in the cells of myometrium or fibroblasts. TGF-β belongs to multifunctional cytokines with promitotic effect and potential to induce synthesis of extracellular matrix resulting in fibrotisation (Arici et al. 2000). Proliferating of smooth muscle cells including myometrium and UF is influenced by basic fibroblast growth factor (bFGF) and we can find it in excess in the extracellular matrix of UF. The effect of epidermal growth factor (EGF) is under the action of progesterone during the luteal phase of menstruation cycle which potentiates its effect. PDGF is a potent mitogen which synergises with other growth factors (Dixon et al. 2000). Although vascular endothelial growth factor (VEGF) is not a mitogen to smooth muscle cells, its effect consists in the stimulation of angiogenesis and an increase in vascular permeability essential to tumor growth. Prolactin was isolated from the myometrium, endometrium and also UF, where its production is probably under the influence of ovarian hormones. Estrogens stimulates prolactin production and thus prolactin has been suggested as a potential growth factor for the cells of both myometrium and UF.

Cytokine gene polymorphism

Genetics and hereditary causes have been considered over recent years and several epidemiologic findings indicate a strong genetic influence. Monozygotic twins have higher incidence of UF compared to dizygotic twins (Treloar et al. 1992; Luoto et al. 2000). First degree relatives of affected probands have a 2.5 times higher risk for developing UF, and the odds ratio increases to 5.7 after selecting for early onset cases (Hodge et al. 2007).

The importance of cytokine gene polymorphism (CGP) and the susceptibility to leiomyoma is still not well understood in the published data. There are only a limited number of studies establishing an association between CGP and leiomyoma.

In a population of 131 Austrian women diagnosed with UF, the association of promoter polymorphism -511 C of IL-1β was observed (70.6% C allele in the patient

group vs 57.1% in the control group, p < 0.0002; odds ratio (OR) 1.81; 95% confidence interval (CI): 1.32-2.47). When a dominant genotype model was applied, homozygous individuals CC were more susceptible to the occurrence of UF (C/C vs. C/T+T/T; P < 0.0002; OR 2.73; 95% CI: 1.77-4.2) (Pietrowski et al. 2009).

Another positive finding was observed in IL-6 promoter polymorphic site -174. This case-control study of 73 Ukrainian women compared the allele distribution of promoter polymorphism and the authors found a significant increase of C allele in the patient cohort when compared to the control group (Litovkin et al. 2007).

The last two published papers describe an association of UF with the polymorphism of IL-12 beta subunit and TNF-α in a Taiwanese population. Hsieh et al observed G allele positivity and homozygosity of GG at codon 378 of IL-12 to be a risk factor for leiomyoma (Hsieh et al. 2007). Allele G at promoter site -308 of TNF-α gene is associated with decreased TNF-α production. Hsieh et al observed higher incidency of leiomyoma among patients with G allele (Hsieh et al. 2004).

Interstitial Lung Diseases

According to the American Thoracic Society (ATS) and the European Respiratory Society (ERS), Idiopathic pulmonary fibrosis (IPF), hypersensitivity pneumonitis (HP) and sarcoidosis belong to the broader group of so called interstitial lung diseases (ILD) which form a subgroup of diffuse parenchymal lung diseases. They are characterised by similar clinical symptoms, physical observation, radiological and pathological image. Not only interstitium suffers from these processes but often bronchi, pleura and lung bloodstream as well. These processes may lead to tissue fibrotisation resulting in irreversible lung fibrosis and finally to worsening of life quality and shortening of the lifespan.

Idiopathic pulmonary fibrosis

Characteristic

IPF is characterised by usual interstitial pneumonitis and progressive interstitial fibrosis caused by excessive extracellular matrix deposition. Regions of fibro-

blast and myofibroblast accumulation, specifically between the vascular endothelium and alveolar epithelium, disrupt the architecture of the lung, giving a "honeycomb" appearance (Chambers et al. 1998; Bogatkevich et al. 2001; Wilson et al. 2009).

IPF affects both genders but men more frequently. The incidence reaches 30/100 000 with 34 000 new cases annually in USA and the number is similar to United Kingdom (Raghu et al. 2006). The numbers increase with age. The common patient's age is in range 50-70 years. We can find a smokers history in the anamnesis. If patient is a smoker, IPF can also manifest at a younger age. IPF is very deep-going illness with a mortality about 50-70% within 5 years from the diagnosis and a mean time survival only 3-5 years (Daniil et al. 1999). The conventional therapy is represented by immunosupressive drug treatment (corticosteroids, azathioprine, cyclophosphamid). Lung transplantation is the only treatment for patients resistant to medical therapy.

Symptoms

The following symptoms are not specific only for IPF and can occur in a wide variety of other pulmonary disorders. Patients mostly suffer with insidously progressing exertional dyspnea (difficulty breathing), dry cough, fever, fatigue, weight loss, arthralgia, 40-75% of patients record clubbing (a disfigurement of the fingers), rales (a crackling sound in the lungs during inhalation), and in later stages also cor pulmonale is common.

Diagnosis

The diagnosis is preferentially aimed on the exclusion of other known causes of interstitial pulmonary processes, e.g. drug intoxication, environmental impacts or connective tissue diseases. Further, we observe abnormal pulmonary function tests with evidence of reduced vital capacity and impaired gas exchange and bibasilar reticular abnormalities with minimal ground glass on high resolution computed tomography (HRCT) scans. The histopathology of transbronchial lung biopsy should show evidence of lung tissue destruction and fibrosis of alveolar septa without inflammatory interstitium impairment. Bronchoalveolar lavage (BAL) testing displays a higher number of neutrophils which correlates with reticular abnormalities, lowered concentrations of phospholipids and surfactant proteins compared to serum, where we can

find higher levels of surfactant protein A and D. As minor criteria, we use age (> 50), insidious onset of otherwise unexplained exertional dyspnea, duration of illness more than 3 months and bibasilar inspiratory crackles.

Mechanisms

Despite extensive investigation, the cause of IPF remains still poorly understood. IPF is a disease characterised by the accumulation of neutrophils in lung alveoli and mononuclear cells in the interstitium following collagen deposition and destruction of a pulmonary tissue. For a long time it has been considered that it is induced by a persistent antigenic stimulation that results in unresolved chronic inflammation which further causes the fibrotic response. It can be either known agents (allergens, toxic chemicals, radiation) or unknown factors that trigger IPF. More recent data suggest that inflammation does not play a major role in inducing the inititation of the disease. IPF seems to be a result of an epithelial/fibroblastic disorder. It has been suggested that epithelial injury and activation of alveolar epithelial cells together with myofibroblast cross-talk represent the key factor.

The role of cytokines in IPF

Animal model showed the enrolment of chemokine/cytokine network capable of modulating the different phases of IPF: namely IL-1, TNF-α, CXC like chemokines: IL-8, epithelial neutrophil-activating protein (ENA)-78, interferon-inducible protein (IP)-10, monokine induced by gamma-interferon (MIG) and interferon-inducible T-cell alpha chemoattractant (I-TAC), CC like chemokines – MCP-1, MIP-1α, thymus and activation regulated chemokine (TARC), macrophage-derived chemokine (MDC) and profibrotic TGF-β and PDGF.

IL-1

IL-1α and IL-1β are widely expressed with potent inflammatory properties. Their expression has been proved in alveolar macrophages from the lungs of IPF patients (Zhang et al. 1993; Pan et al. 1996). Both of them are able to induce a profibrotic phenotype through PDGF, fibroblast proliferation and procollagen type I and type III synthesis (Goldring et al. 1988; Raines et al. 1989). Studies on the animal model have confirmed a role of IL-1β in pulmonary tissue injury and repair. Transi-

ent overexpression causes acute inflammation and tissue destruction in rodents, followed by elevated production of fibrogenic cytokines like TGF-β leading to progressive interstitial fibrosis (Kolb et al. 2001). A further effect of IL-1β resides in the induction of osteopontin expression in fibroblasts (Serlin et al. 2006). Osteopontin is a multifunctional matrix cellular protein upregulated in IPF patients. The role of IL-1β in IPF is supported by the milder disease progress when the inhibition of IL-1β is administrated (Piguet et al. 1993).

TNF-α

Similar to IL-1, TNF-α belongs to inflammatory cytokines with a wide range of biological effects and its expression. It stimulates an inflammatory response by acting on mononuclear cells, netrophils and endothelial cells. TNF-α is produced by activated macrophages and lymphocytes, epithelial cells and endothelial cells. Its central role resides in stimulation of cell-cell adhesion, transendothelial migration and cytokine/chemokine production cascade. It is known that TNF-α directly or indirectly stimulates expression of TGF-β, IL-1, IL-6, IL-8, MCP-1, PDGF, granulocyte-macrophage colony stimulating factor (GM-CSF), triggers fibroblast proliferation and their ability to degrade extracellular matrix.

The presence of TNF-α have been proved in areas of lung fibrosis. In an animal model of lung fibrosis, increased levels of TNF-α were detected and corresponded to increased levels of TGF-β and procollagen type I and III (Zhang et al. 1997). Furthermore, TNF-α knock-out mice fail to develop fibrosis after treatment with bleomycin (BLM) (Liu et al. 1998). Administration of anti-TNF antibody reduces production of TGF-β and IL-5 and eases lung inflammation. TNF-α is abundantly expressed in fibrotic lungs of IPF patients (Zhang et al. 1993). Despite all this evidence of the important role of TNF-α in the pathogenesis of IPF, clinical trials with TNF-α have met with little success (Vassallo et al. 2002; Selman et al. 2004).

TGF-β

It is produced by several cell types with wide array of biological functions including cell growth and differentiation, extracellular matrix (ECM) production, embryonic development, wound healing (Sporn et al. 1992). Isoform TGF-β1 (beside

TGF-β2 and 3) plays a pivotal role in the regulation of lung fibrosis (Broekelmann et al. 1991). Its expression and protein secretion are increased in BLM-fibrosis (Hoyt et al. 1988) and IPF (Khalil et al. 1996). IL-13 mediated overexpression of active form of TGF-β1 results in prolonged and sever fibrosis (Sime et al. 1997; Lee et al. 2001). The action of TGF-β is through recruitment and activation of monocytes and fibroblasts, induction of ECM and stimulation of angiongenesis. In IPF fibroblasts exhibit profibrotic phenotype, with a lower growth rate and increased spontaneous apoptosis (Ramos et al. 2001; Hetzel et al. 2005). TGF-β differentiates fibroblasts to myofibroblasts which represents the main source of ECM during fibrogenesis - TGF-β promotes ECM gene transcription including collagen I, III, IV and V, fibronection and proteoglycans and by suppressing the activity of matrix metalloproteinases (MMP), plasminogen activators and elastases, which results in the inhibition of collagen degradation (Eickelberg et al. 1999; Selman et al. 2000; Ruiz et al. 2003).

PDGF

PDGF family is represented by four subunits: PDGF-A, -B, -C and –D forming disulfide-bonded homo- or heterodimers. The inhibition of their receptors PDGFR-α or –β leads to lung fibrosis attenuation. The source of PDGF are macrophages, fibroblasts, epithelial and endothelial cells. Increased levels of PDGF are characteristic in a BLM-induced animal model of lung fibrosis. Its expression is induced by profibrotic cytokines (TGF-β, TNF-α, and IL-1β) (Raines et al. 1989; Battegay et al. 1990; Battegay et al. 1995; Kolb et al. 2001).

Chemokines

CCL2 (MCP-1) and CCL3 (MIP-1α) belong to proinflammatory chemokines responsible for monocyte recruitment. They are secreted by lymphocytes, macrophages, fibroblasts and endothelial cells. They are both upregulated in BAL of IPF patients and BLM-induced fibrosis (Car et al. 1994; Zhang et al. 1994).

The effect of CCL2 (MCP-1) on the pathogenesis of lung fibrosis has been proved in an animal model: CCR2 knock-out mice were protected from BLM-induced fibrosis (Moore et al. 2001) because expression of fibrogenic cytokine has been impaired as well as fibroblast responsiveness to TGF-β (Gharaee-Kermani et al.

2003). Furthermore, macrophage recruitment was decreased and production of extracellular matrix-remodeling enzymes was reduced (MMP-2 and MMP-9) (Okuma et al. 2004).

CCL17 (TARC), CCL22 (MDC) and their receptor CCR4 are elevated in the areas of fibrotic lung tissue. It is commonly accepted that pathological lung fibroproliferative response is associated with immune deviation toward Th2 immune response. CCL17 and CCL22 are regulated by Th2 cytokines (Belperio et al. 2004). CCR4 is expressed mostly by macrophages and CCL17 or CCL22 neutralisation leads to a reduction of lung damage.

CXC chemokines like CXCL8 (IL-8) or CXCL5 (ENA-78) are elevated in BAL of IPF patients, while CXCL10 (IP-10) level is lowered (Keane et al. 1997; Keane et al. 2001). IP-10 seems to attenuate fibroblast accumulation by limiting fibroblast migration since it has been demonstrated that IP-10 knock-out mice rapidly increases fibroblast accumulation in the lungs (Tager et al. 2004). A blockade of IL-8 and ENA-78 inhibits angiogenic activity in IPF.

CXCL11 (I-TAC) is implicated in IFN-γ dependent negative regulation of fibrogenesis and angiogenesis: administration of exogenous CXCL11 reduces lung damage by inhibition of neoangiogenesis and vascular remodeling (Burdick et al. 2005).

CXC receptor 3 (CXCR3) plays a fundamental role: knock-out animals die more frequently, because IFN- γ production is reduced along with IP-10; CXCR3 thus seems to promote endogenous INF- γ production. CD4+ T cells in BAL expose a CXCR3/CCR4 imbalance in favor of CCR4 and reduce IP-10 (Jiang et al. 2004; Pignatti et al. 2006).

Hypersensitivity pneumonitis

Characteristic

Hypersensitivity pneumonitis (HP), previously known as Extrinsic Allergic Alveolitis (EAA), develops upon exposure to organic dust. Initially it has been described as farmer´s lung, because it was observed frequently in farmers. At present,

several antigens were defined like avian proteins, fungi, termophilic bacteria and low-molecular weight chemical compounds (Table 1-Table 3, (Navarro et al. 2006)). The definition of HP characterises it as a pulmonary disease with symptoms of dyspnoea and cough resulting from the inhalation of an antigen to which the patient has been previously sensitised (Lacasse et al. 2003). Another definition describes HP like an inappropriate immune response to inhaled antigens that causes shortness of breath, a restrictive lung defect, interstitial infiltrates seen on lung imaging (X-ray and HRCT) caused by the accumulation of large numbers of activated T lymphocytes in the lungs.

The exact numbers of prevalence and incidence are unknown. The definition, for example, of well-known farmer´s lung is still not consolidated and thus up to 73% of cases with HP can be misclassified (Kipen et al. 1990). The whole issue is further complicated by climate, geographical conditions or farming practices. However, from several studies with consistent results, we can estimate the prevalence of farmer´s lung to 0.5 – 3 % (Babbott et al. 1980; Marcer et al. 1983; Depierre et al. 1988; Stanford et al. 1990; Dalphin et al. 1993; Ferri et al. 2003). It is estimated that 5-15% of people exposed to high concentration of appropriate inhalant antigen develop HP. Untreated HP can progress into irreversible lung damage.

Table 1 Classification of HP (organic antigen)

Agriculture	Specific antigen	Exposure
Tobacco worker's lung	Aspergillus spp	Moldy tobacco
Wine-grower's lung	Botrytis cinerea mold	Moldy grapes
Bird fancier's lung	Avian proteins	Feathers and bird droppings
Compost lung	Aspergillus	Compost
Farmer's lung	The molds Thermophilic actinomycetes Aspergillus species Saccharopolyspora rectivirgula, and Micropolyspora faeni	Moldy hay

Table 2 Classification of HP (inorganic antigen)

Industry	Specific antigen	Exposure
Woodworker's lung	Alternaria, Penicillium spp	Wood pulp, dust
Cheese-washer's lung	Penicillum casei or P. Roqueforti	Cheese casings
Chemical worker's lung - Isocyanate HP	Toluene diisocyanate (TDI), Hexamethylene diisocyanate (HDI), or Methylene bisphenyl isocyanate (MDI)	Paints, resins, and polyurethane foams
Chemical worker's lung - Trimellitic anhydride (TMA) HP	Trimellitic anhydride	Plastics, resins, and paints
Coffee worker's lung	Coffee bean protein	Coffee bean dust
Detergent worker's disease	Bacillus subtilis enzymes	Detergent
Laboratory worker's lung	Male rat urine protein	Laboratory rats
Malt worker's lung	Aspergillus clavatus	Moldy barley
Sauna worker's lung	Aureobasidium, Graphium spp	Contaminated sauna water
Metalworking fluids HP	Nontuberculous mycobacteria	Mist from metalworking fluids
Mushroom worker's lung	Thermophilic actinomycetes	Mushroom compost

Table 3 Classification of HP (in-house antigen)

Home	Specific antigen	Exposure
Tap water HP	Unknown	Contaminated tap water
Familial HP **Also called Domestic HP**	Bacillus subtilis, puffball spores	Contaminated walls

Symptoms

Classification of HP is defined in three forms: acute, subacute and chronic. Symptoms of ***acute form*** may develop 2-9 hours following exposure to the provoking antigen. Symptoms include febrility, chills, headache, artralgia, myalgia, dyspnoea, cough and chest tightness. Symptoms often resolve within 24-48 hours upon cessation of exposure. The ***subacute form*** develops over days or weeks. It is characterised by cough and dysponea and may progress to severe dyspnoea and cyanosis, leading to urgent hospitalisation. Patients with the ***chronic form*** often lack a history of acute episodes. It has an insidious onset over a period of months. Among symp-

toms we can include increasing cough, progressive dyspnea, fatigue, and weight loss. Histopathological picture includes interstitial lymphocytes infiltrates and fibrosis, oedema, noncaseating granulomas, and bronchiolitis obliterans. Infiltrating macrophages have a characteristic foamy cytoplasm.

Mechanisms

The mechanism of HP was precisely desribed by Suga et al (Suga et al. 1997). The formation of granuloma is triggerd by the T cell-mediated delayed-type hypersensitivity reaction to organic dusts or active chemicals invading the lung. Circulating, antigen-reactive, memory CD4+ T cells, generated by previous sensitisation, migrate into lung parenchyma in response to chemokines such as CCL5 (RANTES). The T cells develop into either Th0, Th1, or Th2 effector depending upon the conditions in which they first encounter the antigens. The Th1 cells produce IL-2 and IFN- γ. IFN- γ can prime macrophages to transcribe and secrete greater amounts of TNF-α and IL-1. The macrophages activated by TNF-α and IL-1 produce a wide range of biologically active mediators such as macrophage activating factor (MAF), macrophage chemotactic factor (MCF), and macrophage inhibition factor (MIF). These monokines attract imature macrophages into the lesions, activate them, and imature macrophages develop into mature macrophages, resulting in the hypersensitivity of granuloma consisting of epithelioid cells and multinucleated giant cells. CD8+ T cells which often exceeds CD4+ T lymphocytes in the lesions of HP, may modulate the granuloma formation via the production of Th1-like or Th2-like cytokines. CD4+/ CD8+ T cell ratio lower than 1 is often observed in HP patients and it is a good marker for diagnosis and for the assessment of disease stage (Trentin et al. 1990). A lower ratio is associated with the chronic form whereas predominance of CD4+ T cells is related to the acute phase (Lacasse et al. 2004; Ismail et al. 2006). This ratio, however, depends on the type and dose of the inhaled antigen (Ismail et al. 2006; Cordeiro et al. 2007).

Antibodies are often found in BAL of HP patients, because sensitisation with antigen also leads to activation of B lymphocytes. Immunocomplexes of antigen and antibody triggers a complement cascade which in turn activates macrophages through the C5 unit (Burrell et al. 1981; Ando et al. 1999). The titre of antibodies can

even correlate with the severity of HP (Ismail et al. 2006). However, there is no proof that they are involved in the pathogenesis of the disease. For example, only 10% of patients with Farmer´s lung develop antibodies against triggering Saccharopolyspora rectivirgula, while ony 0.3 % will get the disease (Cormier et al. 1985). The main mechanism is attributed to the cell infiltration, particularly to lymphocytes.

Role of cytokines in HP

The nature of offending antigens are small slowly, degradable particles retaining in the lungs. Antigen interacts with complement, antibodies and leucocytes, which causes the release of inflammatory mediators (reactive oxygen species, prostaglandins, leukotrienes, IL-1, IL-8, TNF, IL-12, IL-6, MCP-1 and MIP-1α). These cytokines regulate influx of other immune cells, for example, by up-regulation of ICAM-1 expression on alveolar macrophages which in turn upon interaction with T lymphocytes through its ligand lymphocyte function-associated antigen-1 (LFA-1), enhances their antigen-presenting capacity (Popper et al. 2002). Macrophages secreting IL-12 creates a cytokine mileau for Th0-Th1 differentiation. The pathology seems to be more severe when Th1/Th2 balance moves towards a Th1 response.

However, it is suspected that the antigen itself is not sufficient for HP development; we suppose also a role of genetic susceptibility. There are individuals progressing even in the absence of the provoking antigen. There is data in the literature showing an association between TNF-α polymorphism and bird-fancier´s lung or HLA class II antigens and HP (Camarena et al. 2001; Ismail et al. 2006).

Sarcoidosis

Characteristics

Sarcoidosis is a multiorgan disease with an unknown etiology. It affects upper airways, central nervous system, cardiovascular system, musculoskeletal system, gastrointestinal system, liver, kidney, lymphatic system, eyes and skin. The onset of the disease is common for people under 35. There is a second extra risk period for women around 65 (Agostini et al. 1997; Newman et al. 1997; Johns et al. 1999; Vourlekis et al. 2000).

The incidence of sarcoidosis is variable. It depends on the ethnicity and on the geographical location. The incidence ranges from 64 patients per 100,000 population in Sweden to 9 per 100,000 population in Italy with intermediate numbers observed in Denmark (53 per 100,000), Germany (43), Ireland (40), Norway (27), The Netherlands (22), the United Kingdom (20), Switzerland (16) and France (10) (Muller-Quernheim 1998). We can find a decreasing trend in incidence from the North to South. Interestingly, smokers suffer from sarcoidosis less than non-smokers.

Symptoms

Patients often complain on febrility, weight loss, overall weakness, cough, dyspnoea on exertion and chest tightness.

Diagnosis

Diagnosis is based on the HRCT, investigation of blood, BAL and histopathology. Lymphocytopenia in the peripheral blood, increased level of liver tests, hypercalcemia and increased level of serum angiotensins converting enzyme (ACE) are common markers of sarcoidosis. Higher number of neutrophils in BAL often means a worse prognosis. In each case, the investigation of BAL should be accompanied with a biopsy to detect granuloma. Characteristically, we can find lymphocytosis in the lungs compared to the peripheral blood, mainly in the epithelium, interstitium, bloodstream and BAL. We can use the CD4 and CD8 ratio in BAL in the diagnosis. Normally, we find a 2:1 ratio but diseased patients have higher ratio (3-10:1) and in the peripheral blood the ratio is lowered to 1-1.5:1 (normal value is 1.5-3:1) (Agostini et al. 1998a).

Mechanisms

Sarcoidosis evolves on the basis of very difficult degradable antigen stimulus resulting in granuloma formation. There are several supposed antigen stimuli like talc, beryllium, and pine pollen as non-infectious antigen. However, there is also evidence for infectious agents, because they had been cultured from serum, skin lesions or lymf nodes of sarcoid patients, e.g. Yersenia enterocolitica, Borellia burgdorferi, Aspergillus and Nocardia. However, their finding seems to be rather conincidental than casual. Similarly, viral agents like EBV, CMV, HSV, HHV-6, rubella or parainfluenza viruses were detected, but again, unsuccessfuly isolated

from sarcoid tissue (Moller 1997). Although there is no evidence for sarcoidosis of being an autoimmune disease, the role of self-antigen is also suggested. Etiopathegenesis of sarcoidosis is still not well elucidated at present. The antigen itself is not sufficient for the disease development; thus we also suppose a role of genetic susceptibility. There is several data in the literature showing genes like HLA class II, TNF-α, CCR2 and CCR5, IL-2 and finally IFN- γ. The most convincing data has been gathered for IFN- γ because of the correlation of IFN- γ level with the severity and the course of the disease.

The formation of granuloma begins with the accumulation of immunocompetent cells at the sites of inflammatory response to an antigen, further with specific T-cell triggering followed by release of Th1cytokines. Granuloma contains a characteristic central aggregate formed from mononuclear phagocytes, epitheloid cells and mutlinucleated cells surrounded by a rim of CD4+ and CD8+ T lymphocytes together with B lymphocytes. On the periphery, we can also find fibroblasts and collagen fibers (Agostini et al. 1997). The ongoing chronic antigenic stimulation causes alveolar and interstitial edema and disruption of alveolar structures. Parenchymal cells react by physiologic repair and try to restore normal alveolar structures (Agostini et al. 1997). These repair processes are accompanied with exaggerated collagen production associated with fibroblast migration and proliferation and an abnormal increase in the extracelular matrix resulting in fibrosis. The persistent activity of inflammatory cells also leads to fibrosis spreading and affecting the vasculature. The role of Th1 lymphocytes resides mainly in the initial alveolar injury, because in later disease stages, T cells are not usually increased. The fibrotic process is rather modulated by macrophages, neutrophils, eosinophils and mast cells (Bjermer et al. 1987; Inoue et al. 1996).

The role of cytokines in Sarcoidosis

Lymphocytes and macrophage-derived cytokines create a milieu which acts as a local growth factor for T lymphocytes infiltrating the sarcoid tissue. CXCL10 (IP-10), IL-15 and CCL5 (RANTES) cooperate in disease development and progress because they stimulated CD4+ T cell expansion within the granulomatous area (Agostini et al. 1996a; Agostini et al. 1996b; Oshima et al. 1999).

In the early beginning, sarcoid granulomatous areas infiltrating T lymphocytes posess a Th1-profile, because we can detect elevated mRNA and protein levels of IL-2 and IFN-γ (Moller 1999). IL-2 acts as a local growth factor for sarcoid T lymphocytes, while IFN-γ enhances functions of local macrophages, enhances the cytotoxic activity of macrophages and T lymphocytes and finaly regulates secretion of other lymphokines (Muller-Quernheim et al. 1986; Saltini et al. 1986; Konishi et al. 1988).

IL-15 shares several biological activites with IL-2 and shares components of its receptor (β/γ subunits). It is produced mainly by monocyte-macrophages. In activated T lymphocytes it induces cell proliferation, growth and chemotaxis and synergises with IL-12 and IFN-γ. It behaves as a costimulatory factor for GM-CSF and TNF-α, has chemotactic activites and induces production of CC, CXC or C-type chemokines. IL-15 inhibits T cell apoptosis and is believed to be involved in the persistence of inflammation during the chronic sarcoidosis (Agostini et al. 1996a).

Several studies have proved the role of IL-12 and IL-18 (Bergeron et al. 1997; Agostini et al. 1998b; Cameron et al. 1999; Moller 1999) as macrophages from patients with early disease produce high amount of IL-12 and it can be even detected from cultured macrophages from sarcoid patients. Patients with active disease have markedly elevated levels of p40 subunit in BAL (Moller et al. 1996). Sarcoid lung Th1 lymphocytes express a high concentration of IL-12R, a signaling molecule of T cell differentiation (Rogge et al. 1999).

TNF-like molecule family is also considered to be involved in the sarcoid inflammatory processes. There is data from an animal model showing that TNF-α and IFN-γ are essential for granuloma formation. Transgenic mice overexpressing TNF receptor 1 are unable to use TNF-α and show a high susceptibility to mycobacterial infection with vast necrotic lesion in the lung. Furthermore, double knock-out mice in TNF-α and IFNγ form granulomas of large size and heterogenous cellular content.

CXCL10 (IP-10) is an important chemoattractant that modulates the directional migration of activated T cells together with CXCL9 (MIG) and CXCL11 (I-TAC) for which T cells are equipped with the common CXCR3 receptor. While CXCL10 is mainly produced by granuloma macrophages, the latter two cytokines are

produced by epithelial and endothelial cells showing an involvement of non-immune cells in the pathogenesis of sarcoidosis. Interestingly, there is a correlation between CXCL10 levels and lymphocytosis in BAL.

Th1-type cytokines seem to be involved in the initiation of sarcoidosis, but, on the other hand, dominating fibrotic involvement of lung tissue in patients with higher stage of the disease (III. – IV.) also suggests a possible role of Th2 lymphocytes.

The aims of the thesis

Cytokines are the key factors playing an important role both in organ transplantation and ILDs or UF. It has been proved that gene polymorphism has an impact on cytokine expression. The aim of this dissertation thesis was to assess the gene polymorphism of chosen pro- and anti-inflammatory cytokines to find whether the gene polymorphism can have an impact on the transplant outcome or pathogenesis of studied diseases.

1) Characterisation of the impact of cytokine gene polymorphism on the outcome of organ transplantation.
2) Assess the importance of CGP in interstitial lung diseases as a marker of idiopathic pulmonary fibrosis, hypersensitivity pneumonitis and sarcoidosis. Assess the importance of CGPs and their influence on the clinical parameters of these diseases.
3) Examine the importance of CGP in the risk of development of uterine fibroid as a marker of this disease.

List of publications

1. Skorpil N, ***Kolesár L***, Striz I, Lardy NM, Slavcev A. Cytokine gene polymorphisms in the Dutch population. Int J Immunogenet. 2007 Apr;34(2):87-90. IF = 1.279

2. ***Kolesar L***, Novota P, Krasna E, Slavcev A, Viklicky O, Honsova E, Striz I. Polymorphism of interleukin-18 promoter influences the onset of kidney graft function after transplantation. Tissue Antigens. 2007 Nov;70(5):363-8. IF = 2.245

3. Vasakova M, Striz I, Slavcev A, Jandova S, ***Kolesar L***, Sulc J. Th1/Th2 cytokine gene polymorphisms in patients with idiopathic pulmonary fibrosis. Tissue Antigens. 2006 Mar;67(3):229-32. IF = 2.462

4. Vasakova M, Striz I, Slavcev A, Jandova S, Dutka J, Terl M, ***Kolesar L***, Sulc J. Correlation of IL-1alpha and IL-4 gene polymorphisms and clinical parameters in idiopathic pulmonary fibrosis. Scand J Immunol. 2007 Mar;65(3):265-70. IF = 1.928

5. Vasakova M, Striz I, Dutka J, Slavcev A, Jandova S, ***Kolesar L***, Sulc J. Cytokine gene polymorphisms and high-resolution-computed tomography score in idiopathic pulmonary fibrosis. Respir Med. 2007 May;101(5):944-50. IF = 2.235

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7. Sosna O, ***Kolesar L***, Slavcev A., Skibova J, Fait T, Mara M, Striz I, Kuzel D; Th1/Th2 cytokine gene polymorphisms in patients with uterine fibroid. Folia Biol (Praha). 2010;56(5):206-10. IF = 0.729

Comment to article 1

Cytokine gene polymorphisms in the Dutch population

We can find a great heterozygosity in results comparing genetic polymorphisms and clinical parameters of various diseases in the literature. We assume a great influence of sample size – small patient numbers with inadequate power to detect a difference, sampling method – inadequately defined end points, and last but not least the genetic background. Differences in the allelic distribution between certain ethnicities have been well described and we can find specialised databases on the web such as http://www.allelefrequencies.net. To confirm this observation, we set up a study comparing the allele and genotype distribution of 13 pro- and anti-inflammatory cytokine genes among selected European populations – the Czech, the Dutch and the Italian.

Our results have shown that 3 out of 22 tested SNP differed in allele frequency when comparing the Italians x the Dutch or the Czechs x the Dutch. Four out of 22 SNP differed in genotype frequency between Italian and Dutch population and 3 between Czech and Dutch population. Genes with different distribution were, namely, IL-1α, IL-1β, IL-1R, TNF-α, IL-2 and IL-10.

Conclusion: Our results might be useful for the analysis of the occurrence and progression of diseases, presumed to be influenced by variation of cytokine production. Furthermore, our results also confirmed the previous findings that it is necessary to take into account the genetic background of the tested population when considering the effect of polymorphism on a clinical end point. A certain polymorphism can lead to the susceptibility to a disease or a higher gene expression in a particular population but it cannot be easily applied to another population.

Comment to article 2

Polymorphism of interleukin-18 promoter influences the onset of kidney graft function after transplantation.

In our previously published data, we showed constitutive IL-18 expression in the epithelium of renal distal tubules in patients after kidney transplantation and significantly elevated IL-18 expression during acute rejection. Furthermore, we have shown that in addition to immune cells, renal epithelial cells are a significant source of IL-18. In addition, the functional promoter polymorphism influencing the gene expression has been described.

In this study, we evaluated the clinical significance of two SNPs of the IL-18 gene at positions -607 A/C (rs1946518) and -137 C/G (rs187238) in patients after kidney transplantation and looked for associations with the onset of graft function and the incidence of rejection episodes.

Analysis of allele and genotype frequencies in a group of 124 patients and 103 unrelated controls revealed a statistically different distribution of the alleles of SNP -607 A/C between patients with immediate or delayed onset of kidney graft function. Data showed that the C allele, which contributes to higher IL-18 expression, is more frequent in patients with delayed onset of function ($p = 0.03$, OR = 1.93; 95% CI = 1.15-3.25).

Conclusion: Our data support the theory of the important role of IL-18 in the early immune response after transplantation. Together with our finding, a genetic predisposition of higher IL-18 production could contribute to an elevated risk of delayed graft function after transplant.

Comment to article 3

Th1/Th2 cytokine gene polymorphisms in patients with idiopathic pulmonary fibrosis.

Idiopathic pulmonary fibrosis (IPF) is a deleterious disease with grim prognosis regardless of any known treatment. There is little known about the etiology of this disease. The role of cytokines is supposed and a possibility of the role of CGP in its pathogenesis was investigated in some previous studies. The aim of our study was to investigate the large spectrum of Th1/Th2 cytokine gene polymorphisms in patients with IPF.

In this initial work, we analysed 22 SNPs corresponding to 13 different pro-inflammatory and anti-inflammatory cytokine genes on a cohort of 30 patients from the Pulmonary department at the Thomayer Faculty Hospital.

For the majority of SNP, we did not find an association with the risk of IPF. However, we observed a strong correlation of a genotype with the disease at the promoter region of IL-4, where the CC genotypes at the positions -590 and -33 were less frequent in the IPF group ($p < 0.0001$, $p_{(corr)} < 0.0022$; resp. $p < 0.0001$, $p_{(corr)} < 0.0022$).

Conclusion: Our preliminary results support the idea of the pathogenic role of cytokine gene polymorphisms in the etiology and pathogenesis of IPF, with an emphasis on the IL-4 promoter gene polymorphisms.

Comment to article 4

Correlation of IL-1alpha and IL-4 gene polymorphisms and clinical parameters in idiopathic pulmonary fibrosis.

In the previous study, we found a potential association of IL-4 promoter polymorphism with IPF. We were thus encouraged to continue with the analysis of IL-4 polymorphism and its probable implications to clinical parameters in IPF.

The correlations of vital capacity (VC) and diffusing capacity for carbon monoxide (DL(CO)), bronchoalveolar lavage (BAL) fluid cell counts and high resolution computed tomography (HRCT) alveolar and interstitial scores with different genotypes of IL-4 at -1098, -590 and -33 positions and IL-1 alpha at -889 position were tested on a group of 30 patients.

Although CC genotype of IL-1A -889 is less frequent among IPF patients (the difference is not statistically significant), the pulmonary VC of these patients reached lower values at the time of diagnosis. Based on measuring CD3+HLADR+ T lymphocytes in the BAL fluid, the frequency of activated T lymphocytes was higher in CC homozygotes which could support the hypothesis of a potential role of C allele on activation of T lymphocytes in IPF.

From the previous publication, it might seem that CC homozygozity at IL-4 -590 and -33 might have a protective role against IPF. This is supported by our observation that CC homozygotes (SNP -33) were associated with a better HRCT score, i.e. stable form of the disease. A correlation with VC and DL(CO) and BAL fluid cell numbers were not proved.

From the results of BAL fluid analysis, we have found an association of TG genotype of IL-4 -1098 and higher frequency of CD4+ T and lower frequency of CD8+ T compared to TT homozygotes, leading to a statistically significant higher CD4+/CD8+ T ratio of TG heterozygotes.

Conclusion: On the basis of our results, we announce the suspicion of the pathogenic role of the IL-1 and IL-4 promoter gene polymorphisms in IPF development and also of the disease-modifying role of these polymorphisms. We have to emphasise that the group of patients was small and we cannot know in which disease stage patients came to their physician.

Comment to article 5

Cytokine gene polymorphisms and high-resolution-computed tomography score in idiopathic pulmonary fibrosis.

Based on our previous finding of the association between IL-4 and HRCT score, we compared HRCT alveolar and interstitial score, a marker of disease stage and progression, with IL-1, IL-4, IL-12, IL-1RA and IL-4RA cytokine gene polymorphisms in a group of 27 patients.

We found that the higher alveolar score, i.e. a greater extent of active changes at the time of diagnosis, is in IL-4RA(+1902) AG heterozygotes. The lower interstitial score, i.e. the less extent of the fibrotic changes, was found in the IL-12 (-1188) AA homozygotes compared to AC heterozygotes. According to the progression of the disease in serial HRCT investigations, the CC IL-1RA (mspa 111100) homozygotes had a lower grade of progression of the interstitial score (i.e. fibrosis) than the CT heterozygotes and TT homozygotes. Similarly, the IL-4RA (+1902) AA homozygotes had lower progression of the interstitial score than the AG heterozygotes and CC IL-4 (-33) homozygotes had lower interstitial score progression than the CT heterozygotes at this position.

Conclusion: We assume from our data that the polymorphisms of IL-4, IL-4RA, IL-1RA and IL-12 genes (genes of cytokines with regulatory activity) might influence the phenotype of IPF as shown by measurable changes in HRCT investigations.

Comment to article 6

Cytokine gene polymorphisms and BALF cytokine levels in interstitial lung diseases.

In the current study, we aimed to investigate correlations of TH1/TH2 cytokine gene polymorphisms and BALF cytokine and chemokine levels in patients with interstitial lung diseases. We hypothesise that the polymorphisms, especially of regulatory cytokines, could influence the expression of cytokines and chemokines in lung tissue and BALF and thus cause the alternative activation of alveolar macrophages (AMs), which could lead to aberrant wound healing with excessive fibroproduction.

In 16 sarcoidosis, 7 IPF and 8 HP patients, we evaluated IL-1α, -1R, -1RA, -2, -4, -4Rα, -6, -10, -12, IFN-γ, TGF-β1 and TNF-α gene polymorphisms in peripheral blood. Furthermore, we assessed levels of MCP-1,MIP-1α, MIP-1β, RANTES, ENA-78, fibroblast growth factor (FGF), granulocyte colony stimulating factor (G-CSF), GM-CSF, IFN-γ, IL-1α, IL-1RA, IL-1β, -2, -4, -5, -6, -8, -10, -17, TNF-α, thymopoietin (Tpo) and VEGF in BALF.

Although the level of TNF-α did not differ among the patient subgroups, when we compared the level of TNF-α with the cytokine genotypes regardless the diagnosis we found higher TNF-α values in IL-1R pst 1970 TT homozygotes ($p=0.0126$). In the sarcoidosis group, IL-4Rα (+1902) AA and IL-10 (-1082) G allele correlated with higher BALF ENA-78 levels ($p=0.0258$, $p=0.0230$). In the HP group, the IL-6 (-174) CG and IL-6 (nt565) AG correlated with higher ENA-78 BALF levels ($p=0.0253$). In the IPF group, the IL-1β +3962 CC homozygotes had lower IL-1RA BALF values ($p=0.046$). BALF chemokine values did not differ between ILD subgroups, except for IL-8, which was higher in stage III sarcoidosis patients compared to stage I ($r = 0.574$. $p < 0.05$).

Our results show a probable influence of gene polymorphisms, namely IL-4R α and IL-10 on ENA-78 BALF levels in sarcoidosis, IL-6 on ENA-78 BALF levels in HP and IL-1β on IL-1RA BALF values in the IPF group. The TNF-α BALF values correlated with IL-1R pst 1970 gene polymorphisms.

We suppose that cytokine gene polymorphisms, especially of so-called regulatory cytokines, probably influence cytokine mRNA expression and thus also the level of secreted protein and its functional status (activity). The changes in cytokine levels could then influence chemokine levels in target tissues and organs, i.e. lung, and form a milieu enhancing inflammation and granuloma formation, or on the other hand, excessive fibroproduction in answer to an unknown probably mostly common stimuli.

Comment to article 7

Th1/Th2 cytokine gene polymorphisms in patients with uterine fibroid.

Uterine fibroid (UF) is a frequent nonmalignant tumor with unknown etiology and pathogenesis. Cytokines play an important role in the pathogenesis of this disease.

The aim of our study was to look for possible genetic markers which could be used as prognostic tools for evaluation of an increased risk for development of UF.

Within the large spectrum of CGPs studied in a cohort of 102 patients with UF, we came to the major finding that the polymorphisms of the IL-4 gene promoter at positions -590 C/T and -33 C/T was associated with the risk of UF. The CC genotype at these SNPs was less frequent in studied patients than in the control group (corrected $p = 0.03$). Besides IL-4, we observed higher proportion of AA genotype (-308A/G.) of TNFA gene in the younger (<35 years) patient group ($p=0.02$).

Conclusion: Our study suggests that certain cytokine gene polymorphisms, especially of the IL-4 and TNF-α genes may be associated with increased risk for development of uterine fibroid. Further investigation would be needed for elucidation of the mechanisms responsible for these associations.

Discussion and conclusions

The aim of the dissertation thesis was to analyse cytokine gene polymorphisms as genetic markers of a disease or as markers which could influence or serve as a prognostic information for the outcome of kidney transplantation. Our results indicate that CGP could be associated with these clinical units to a certain extent. We have summarised the following findings:

(1) Ethnicity or even nationality plays a role in the distribution of genetic polymorphism. This must be absolutely taken into account when one would like to transfer findings of a clinical study from a certain nation or ethnic and applied them to his studied group for the comparative purposes (Skorpil et al. 2007).

(2) Our first clinical gene-association study has found that even genetic polymorphism of the IL-18 gene may contribute to the onset of kidney graft function after transplantation. This finding is in agreement with our previous reports on IL-18 production by renal epithelial cells, which support the role of this proinflammatory cytokine in the modulation of early immune responses against the kidney allograft (Kolesar et al. 2007).

(3) From the studies of ILDs, we conclude that cytokine gene polymorphisms contribute to the pathogenesis and play a role in the etiology of ILDs, with an emphasis on the IL-4, IL-4RA, IL-1RA, and IL-12 promoter gene polymorphisms. Associations have been found between the polymorphisms and functional parameters of IPF and between polymorphisms and levels of other cytokines. (Vasakova et al. 2006; Vasakova et al. 2007a; Vasakova et al. 2007b; Vasakova et al. 2009a; Vasakova et al. 2009b).

(4) Finally, as cytokines play a role in the development of UF it seems that their gene polymorphisms play a role as well as we demonstrated on the polymorphism for IL-4 and TNF-α (Sosna et al. 2010).

(1) In the literature, we can find a lot of different reports dealing with cytokine gene polymorphisms in combination with disease diagnosis or disease prognosis trying to find out how genetics can help to answer questions rising from observations of differences among patients having the same disease and same treatment. Unfortunately, in many cases there are ambiguous results. These ambiguities arise from different reasons. We assume that a great influence belong to the size of the studied cohorts – small patient numbers with inadequate power to detect differences. However, in several cases, the assumption of well-sized patient population cannot be satisfied due to the prevalence of the disease, accessibility to patients or merely financial limitations of the project. Other variations come from differences in disease definitions, differences in genetic background of various ethnics or nations, and many others factors which bring about this result ambiguity. Our results have clearly showed that genetic data from one nation cannot be transferred and compared easily with other nation. Marder et al has clearly summarised this statement in table 1 showing positive and negative associations of various CGPs with organ transplantation among different populations (Marder et al. 2003). We are aware that the design of our studies meets some of the above mentioned problems.

(2) But, how can be CGP useful from the clinical point of view? Can we stratify patients according to the certain allele in the specific gene and claim that this group of patients is in a higher risk of rejection progress? According to many contradictory data in the literature it is hard to answer this question. Results of our first clinical study did not bring too much light to this issue. If we come back to our study Kolesar et al. 2007, our main finding was that patients with the C allele at promoter position -607 of IL-18 gene tend to have a delayed graft function (DGF) after transplantation as compared with patients without DGF. We also showed a trend toward high-producing genotype (-607 CC) and CG haplotype (positions -607 and -137, respectively) in these patients, but the differences did not reach statistical significance. We could not prove that patients having a certain allele are prone to rejection episode. This was also shown in a big retrospective study from our transplant centre where all tested CGP failed to find an association with graft rejection (Brabcova et al. 2007). Our data have rather showed that IL-18 is an interesting molecule to study, as we can follow in the literature (Daemen et al. 1999; Melnikov et al. 2001; Parikh

et al. 2004; Simon et al. 2004; Parikh et al. 2006), and that the role of one CGP might not influence such a complicated long process of rejection which includes also risk factors like donor and patient age, number of HLA mismatches, surgical complications or DGF. The take home message from our study is to use IL-18 CGP as a tool of risk definition in smaller processes that confer to graft rejection, like DGF, which do not have to lead to the rejection process in the end. Our data support the theory of the important role of IL-18 in the early immune response after transplantation which we also support with the findings from our previous study (Striz et al. 2005) and contributed to current knowledge of pathophysiological mechanisms of graft rejection.

(3) Previous study was rather aimed at using CGP as a prognostic marker in transplantation setting which means combining two genetically different cells lines (donor allograft versus recipient immune system). Further studies were aimed at usage CGP as a diagnostic tool of 3 different interstitial lung diseases.

Our initial study investigating a wide spectrum of CGP in association with IPF revealed a possible relation with IL-4 polymorphism (Vasakova et al. 2006). These results support the fact that IPF is a disease with suspected Th2 type cytokine prevalence and support the idea of the pathogenic role of the IL-4 promoter polymorphisms in IPF which is in agreement with the study of Jakubzick et al. (Jakubzick et al. 2004) describing the influence of IL-4 on lung cells, especially on fibroblasts. However, the functional consequences of the described IL-4 polymorphisms, i.e., whether these polymorphisms influence the amount of produced IL-4, or induce changes of its affinity to the IL-4 receptors on lung fibroblasts, are not known. G instead of T at position (-1098) of the IL-4 promoter, compared with our results in IPF, was also found in patients with juvenile idiopathic arthritis and could be the basis for the Th1 and Th2 bias of these two diseases (Cinek et al. 2004). The presence of CT or TT at position -590 of IL-4 is cited in connection with rheumatoid arthritis (RA) severity and might reflect the prevailing Th2 reaction in the most severe forms of RA (Pawlik et al. 2005). We must, however, mention that our group of patients was too small to obtain results with high level of evidence.

Our further investigation logically continued to explore if this gene association can have an impact on clinical manifestation of IPF. Our results suggest that

the patients with GG or GT rather than TT genotype at the position -1098 of the promoter region of IL-4 were prone to have higher counts of CD4+ T lymphocytes in BAL fluid ($p = 0.0598$). Inversely, the TT homozygotes at the same position had significantly higher levels of CD8+ T lymphocytes in BAL fluid ($p = 0.0068$). The CD4/CD8 ratio in BAL fluid was higher in the GG or GT versus TT genotype carriers at this position ($p = 0.0516$) (Table 5 in (Vasakova et al. 2007b). These results suggest the possible role of allele G for CD4/CD8 profiling in BAL of IPF patients and may support the idea of protective higher CD4+ lymphocyte counts in BAL against IPF development. But this hypothesis is only speculative based on the finding of a higher prevalence of T allele in patients with IPF compared with healthy controls.

High Resolution Computed Tomography (HRCT) of the lung is a medical tool used for diagnosis and assessment of interstitial lung disease. It involves the use of special computed tomography scanning techniques to assess the lung parenchyma. In our study, according to dynamic changes of HRCT interstitial score over time (at the time of diagnosis and 12 months later), the carriers of CT genotype at IL-4 -33 had more frequently a progressive IPF disease ($p < 0.08$) (Table 6 in (Vasakova et al. 2007b). When we compare this result with our previous findings we might suppose the protective role of C allele either against a development of IPF and against the rapid progression of the disease described as a progression of interstitial score at serial HRCT investigations. Next to IL-4 CGP we also observed higher alveolar HRCT score, i. e. greater extent of active changes at the time of diagnosis, in IL-4RA (+1902) AG heterozygotes as compared with AA genotype (Vasakova et al. 2007a). On the other hand, a less severe HRCT interstitial score, i. e. less extent of fibrotic changes, was associated with IL-12 (-1188) AA genotype. However, we observed no association between IL-12 CGP and susceptibility to IPF which is in accordance with Latsi et al. (Latsi et al. 2003). Finally, CC carriers of IL-1RA mspa 11100 polymorphism showed a greater probability of having a stable disease without a progression of the interstitial score.

Vital capacity (VC), besides body plethysmography or diffusing capacity for carbon monoxide (DLCO), is a good diagnostic pulmonary function test for

ILDs. In IPF patients, we observed a reduction in the VC with either a proportionate reduction in airflows, or increased airflows for the observed VC. We have observed that the VC was statistically significantly higher in the patients with genotype CT or TT rather than CC at the position -889 of the promoter region of IL-1α ($p = 0.0142$) suggesting a protective role of CC homozygozity. The contradictory results of our previous and recent findings may reflect the small number of patients in the clinical subgroups and the fact the T allele was more frequently found in both homozygous and heterozygous form in IPF in our previous study. Secondly, we suppose that the VC at the time of the diagnosis is influenced by many other facts including the patients´ decision when to attend their physician (i.e. some of the patients visited their physicians in an advanced stage of the disease).

Moreover, the carriers of CC at the same position of IL-1α gene had higher counts of HLADR+ T lymphocytes in BAL fluid than CT or TT carriers ($p = 0.0284$) (Table 4 in (Vasakova et al. 2007b). According to CD3+HLADR+ T lymphocytes in BAL, the presence of the CC genotype at -889 position of IL- 1α was more frequently seen in patients with higher counts of these activated T lymphocytes in BAL. This finding might support the hypothesis of the role of the allele C at this position in lymphocyte activation in IPF, but it should be further tested in a larger group of patients.

We are aware that cytokine gene polymorphisms are obviously not the only factor influencing the IPF development and progression and could be a result of the synergic effect of manifestation of variation of cytokine genes and imbalance in tissue remodelling mediators. We suppose that cytokine gene polymorphisms, especially of so-called regulatory cytokines, probably influence cytokine mRNA expression and thus also the level of secreted protein and its activity. The changes in cytokine levels could than influence chemokine levels in target tissues and organs, i.e. lung, and form a milieu enhancing inflammation and granuloma formation, or on the other hand, excessive fibroproduction in answer to unknown stimuli.

Our last study concerning cytokine gene polymorphism and ILDs was a project aimed at the effect of gene polymorphisms on the level of cytokines. We have found that the cytokine BALF values did not differ significantly between the differ-

ent ILD groups. We suppose that this finding could be ascribed to the greater number of patients with advanced, i.e. fibrotic, disease in HP and sarcoidosis groups. Only VEGF, IL-8, ENA-78 and IL-1RA were detected in BALF in all of the patients. When correlating the gene polymorphisms with BALF cytokine levels IL-4Ra (+1902) AA and IL-10 (-1082) G allele correlated with higher ENA-78 levels ($p = 0.0258$, $p = 0.0230$) in the sarcoidosis group (Tables 3 and 4 in (Vasakova et al. 2009b). ENA-78 and IL-8 BALF levels in IPF patients were found to be significantly higher compared with sarcoidosis patients in the study of Antoniou et al. (Antoniou et al. 2008). In the study of Sugiyama et al, ENA-78 BALF levels in patients with sarcoidosis were significantly higher than those in control subjects and were more increased in stage III sarcoidosis, showing that ENA-78 may be associated with lung parenchymal disease in pulmonary sarcoidosis (Sugiyama et al. 2006). This could prove the influence of genetically based IL-10 up-regulation on increased ENA-78 levels and thus on poor clinical outcome. Unfortunately, the results of ENA-78 BALF values in the different sarcoidosis stages did not support this idea in our study. Nevertheless, we might suppose that the clinical course of the disease in those patients with higher ENA-78 might be less favourable, but to date we have not carried out a longitudinal study.

Grutters et al, hypothesised that the IL-6 -174 C allele might play a role in sarcoidosis severity or a progression towards pulmonary fibrosis in a particular subgroup (Grutters et al. 2003). In our study, the IL-6 polymorphisms (IL-6 -174 CG and IL-6 nt565 AG) correlated with higher ENA-78 BALF levels only in the HP group ($p = 0.0253$ for both) which showed the possible role also of IL-6 polymorphisms on ENA-78 production and thus on the prognosis in HP (Tables 5 and 6 in (Vasakova et al. 2009b). Nevertheless, we should be aware of the low number of HP patients in our study.

Shimoji et al found that IL-1RA levels were decreased in healthy smokers as well as IPF and sarcoidosis patients, compared to healthy non-smokers, and suggested that decreased expression of IL-1RA gene may contribute to the development of chronic low-grade inflammation of the lung (Shimoji et al. 1993). In our study, in the IPF group the IL-1b +3962 CC homozygotes had lower IL-1RA values in BALF ($p =$

0.046), which could support the hypothesis of the IL-1 genes group role in the pathogenesis of this disease.

(4) Our last study focused on studying cytokine CGPs as a marker of uterine fibroid. The role of various cytokines in UF development is obvious as it has been investigated in EGF (Shimomura et al. 1998), TGF-β (Arici et al. 2000) or chemokines and chemokine receptors (Syssoev et al. 2008). Besides, it is known that UF growth is dependent on oestrogen and progesterone production (Maruo et al. 2003) which from their parts may influence the gene expression of several cytokines and growth factors. The main finding of our study was a possible association between the polymorphisms of the IL4 gene promoter, namely SNP -590 C/T and -33 C/T, and the risk of UF development. T allele substitution at position -590 increases IL4 gene expression and is associated with elevation of serum IL-4 concentrations (Rosenwasser et al. 1997). On the contrary, the C allele at this position leads to decreased gene expression. It is a matter of speculation as what might be the reason for the observed association. As a strong anti-inflammatory cytokine, IL-4 has been shown to modulate the activation of tumour-associated fibroblasts (Blankenstein 2005). Its role in tumour clearance and reduction of tumour load has also been investigated (Tepper et al. 1989; Golumbek et al. 1991; Hock et al. 1993; Hock et al. 1994). The study of Hsieh et al. (2007) tested the same IL4 -590 polymorphisms and other SNPs; however, no association with the incidence of UF was found with the exception of IL-12Rβ1. This contradiction may be caused by differences between the patient groups studied (premenopausal Taiwanese women) or by a racial difference as it was previously shown in the study of Skorpil et al. (Skorpil et al. 2007). Our data cannot, however, explain the mechanism responsible for these associations and thus further investigation would be needed to elucidate that.

Based on the literature, it is obvious that gene polymorphisms play a role in affecting gene expression. Our data and data from the literature suggest that the gene polymorphism can influence immune reaction. However, the strength of this influence is still unknown. Processes in organ transplantation or in disease pathogenesis are so polygenic and complex and in many cases there is no clear definition what leads to the disease, that it is hard to imagine that one point substitution in the certain

gene could affect so many biochemical and signalling pathways. We have to take into account also a role of epigenetics on the level of gene expression which also seems to play a significant role.

Research of gene polymorphisms means to enroll large cohorts of patients to be able to conclude results with the significant power and to be able to follow interactions among genes which in many studies cannot be fulfilled because of, for example, the low frequency of the disease. Present technologies allow studying thousands of SNPs, reading the whole human genome in couple of days or assess the gene expression just in one cell. With these tools we will be able to understand better the genetic background in the future.

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