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TEENAGERS AND YOUNG ADULTS WITH ASTHMA AND ALLERGY, RISK-FACTORS AND T-CELL REGULATION

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ASTHMA AND ALLERGY IN TEENAGERS AND YOUNG ADULTS RISK-FACTORS AND T-CELL REGULATION

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ABSTRACT

Asthma is one of the most common chronic diseases among teenagers and young adults. The prevalence of asthma among young adults in Sweden is approximately 7-10%. Despite this, only a limited number of studies have focused on asthma, allergy and allergic inflammation in this age group. The aims of this thesis are to study the consequences of asthma and allergy in teenagers and young adults, incidence and risk-factors for death due to asthma, and deterioration in asthma prior and following transfer from pediatric to adult health care. As allergic inflammation is involved in a majority of asthma patients in this age-group we have further investigated a T cell mediated inflammatory mechanism with possible implications in monitoring and modulating autoimmune and allergic diseases.

PAPER I

During the 1994-2003 period 37 deaths due to asthma were identified. The incidence of asthma in 1-34 year-olds decreased during the period from 1.54 to 0.53 per million. Common risk-factors were under-treatment, poor adherence to prescribed treatment and adverse psychosocial situation. An alarming finding was that 11/37 deaths was probably caused by food allergy and 8/37 were associated with exposure to pet dander.

PAPER II

In a 5-year prospective follow-up study to identify risk factors for deterioration of asthma following transfer from pediatric to adult health care 150 teenagers with asthma were enrolled. Skin prick test at entrance revealed that 89% were sensitized towards at least one of tested allergens. A minority performed with impaired lung function without deterioration during the five-year follow up, while bronchial hyper responsiveness (BHR) was present in 71% of the subjects at entrance and among 59% at follow-up. Risk for persistence of BHR after five years was elevated by poor adherence and attenuated by regular physical activity. Working capacity decreased significantly during the study period without any correlation to risk factors examined.

Paper III

Interactions between the low-density lipoprotein receptor-related protein 1(LRP1) and thrombospondin-1 (TSP-1) is necessarily for T cell motility and that the motogenic LRP/TSP-1 mechanism antagonizes adhesion to ICAM-1 and fibronectin as well as TCR induced proliferative responses. This cascade mediates regulatory effects of IL-2 and IL-4. In addition expression of TSP-1, with known ability to protect against inflammation, was increased by IL-2.

Paper IV

T cell activation induces arrest of T cell motility through down-regulation of LRP1 synthesis a concomitant up-regulation of TSP-1 synthesis providing a mechanism for enhancement of adhesion of T cells to APC´s stimulating proliferative responses. Despite this arrest of motility, co-ligation with CD28 maintains a basal motility level by enhancing transport of LRP1 to the cell surface**.**

Paper V

Patients with allergy and psoriasis showed impaired T cell motility and decreased TSP-1 expression compared to healthy controls. IL-2 was shown to up-regulate the impaired motility in patient to the same level as in controls indicating a reversible state probably excluding a constitutional defect.

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CONTENTS

LIST OF ABBREVIATIONS

APC	Antigen presenting cell
BAL	Bronchoalveolar lavage
BHR	Bronchial hyperresponsiveness
BMI	Body mass index
CAM	Cell-adhesion molecule
CTLA-4	Cytotoxic T-lymphocyte antigen
CCR	CC chemokine receptor
CXCR	CXC chemokine receptor
EIA	Exercise induced asthma
FEV1	Forced expiratory volume in one second
FN	Fibronectin
FVC	Forced vital capacity
GlyCAM	Glycosylation-dependent cell adhesion molecule
HEV	High endothelial venules
ICAM	Intercellular adhesion molecule
Ig	Immunoglobulin
IL-	Interleukin
IS	Immunological synapse
JAK	Janus family of tyrosine kinases
LDL	Low density lipoprotein
LFA-1	Leukocyte function associated protein
LRP	LDL receptor related protein (CD91)
MHC	Major histocompatibility complex
PD	Programmed cell death
PLL	Poly-L-lysin
RAP	LDL receptor-associated protein
RT-PCR	Reverse transcriptase polymerase chain reaction
SDF-1	Stromal cell-derived factor 1α
SDS-PAGE	SDS polyacrylamide gel electrophoresis
TCR	T cell receptor
TGF-β	Transforming growth factor beta
Th	T helper cell
TNF	Tumor necrosis factor
TSP-1	Thrombospondin-1

1 INTRODUCTION AND BACKGROUND

1.1 ASTHMA

Asthma, is a multifactorial disease characterized by variable airflow obstruction, bronchial hyperresponsiveness airway inflammation and not seldom airway remodelling[1]. During recent years it has become evident that the disease called asthma includes a number of different wheezing phenotypes e.g. exercise induced asthma (EIA), allergic asthma or unspecific hypersensitive asthma. These different phenotypes have usually specific inflammatory characteristics.

1.1.1 Prevalence of asthma

The prevalence of asthma among teenagers and young adults in Sweden is approximately 7-10%, depending on criteria employed, which is similar to most western countries[2-6] . As a consequence, asthma is one of the most common chronic diseases in this age group. Several studies have reported an increase in the prevalence of asthma the 1960s and the 1980s-1990s which appear to have plateaued in recent years[7].

1.1.2 Asthma in teenagers and young adults

Despite the fact that this age-range represents an important period in life the number of studies focusing on this age group is limited. As a consequence, much knowledge about teenagers and young adults with asthma is obtained by extrapolating results from studies on children and young adults.

1.1.2.1 Psychological consequences and adherence to treatment

The period in life when entering adulthood involves important psychological changes for the adolescent as at the same time the teenager/young adult strives for emancipation and must make decisions of importance for his/her future life. This period also includes a desire for independence and autonomy. These circumstances may be in conflict with the knowledge that asthma-related morbidity can be largely preventable by effective self-management[8-11] . Earlier studies have reported unintentional error such as "forgetfulness" as a common barrier to asthma management[10, 12, 13] . However, others suggest that barriers to adherence could be more of an intentional and active nature with reluctance to comply with medical advice, "trying to forget"[10] . In addition there is a gender aspect in that males with severe asthma deny their chronic disease to a higher extent compared to females[10] . Patients with poor socioeconomic standard and neurocognitive dysfunctions are less likely to have well-controlled asthma[10, 14, 15].

1.1.2.2 Transition from child to adult health care

In Sweden, transfer from paediatric to adult health care usually occurs when the patient is about 18 years of age which is a period when several decisions of great importance for future life have to be taken. In addition transfer to adult health care includes usually that he young adult has to take responsibility for their disease. Together with physiological changes, psychosocial impact during the adolescent period is supposed to have consequences on the outcome of chronic diseases as mentioned above. The need

of a planned transition to adult health care is exemplified in that despite having the lowest rates of hospitalisation, the mortality rate from asthma is 3-times higher for youths aged 15-19 years than for children aged 5-9 years[16-22] . According to Blum et al medical transition is the *"purposeful, planned movement of adolescents and young adults with chronic physical and medical conditions from child-centered to adult-oriented health care systems"* [17]. A special matter of discussion in Sweden is whether patients with asthma should be transferred to an asthma clinic or to primary health care system.

1.1.2.3 Longitudinal studies

Over the last three decades, the reported prevalence of atopy and allergic diseases has increased dramatically in many countries[23 3]. Evidence generated from a large number of cohort studies demonstrates that wheezing that begins in early life and continues into school age generally persists into adulthood[4, 6, 24]. The natural history of asthma in teenagers and young adults is often characterized by periods of remission for a sustained period of time. In a study from New Zealand approximately 30% of young adults with a history of childhood asthma who are in remission at 18 years of age will relapse by 26 years of age which is in agreement with similar studies from other countries[25, 26] . The likelihood of relapse has been reported to be associated with atopy, smoking and previous BHR. In addition, lung function growth patterns established at early school-age generally continue into middle adulthood[4, 24, 27]. A general finding is a 10% decrease in FEV1 and a 5% decrease in FEV1/FVC ratio during early to middle adulthood in those with persistent or relapsed wheezing[4]. The seriousness of persistent severe asthma is exemplified by Limb et al shoving that this subgroup enters adult life with a mean FEV1 of 66% of predicted[28, 29]. Predictors of persistent moderate to severe asthma are early deterioration of lung function, high serum IgE and persistent cough/mucus production[30].

1.1.3 Asthma mortality

Asthma mortality increased, according to several studies between the 1960-80`s in most industrial countries, Sweden included[31-34]. This increase was initially most pronounced among adolescents and young adults but later on also in younger ages[31]. In addition, a tendency was observed in that asthma death not only occurred among those with severe asthma but also in the subgroup classified as mild or moderately severe asthmatics. No single factor explained that this increase in asthma mortality was pin-pointed as the triggering mechanism but increased incidence in allergic diseases, indoor pollution, patient compliance and insufficient anti-inflammatory treatment was suggested as possible causes[31, 32].

Studies of mortality due to asthma in children and young adults have revealed decreasing rates in most European countries as well as in the United States during the two last decades[33, 35, 36]. Despite this, asthma mortality is still reported to remain the sixth leading cause of death of children between 5 and 14 years in the Unites States. A recent prospective study from Denmark showed an increased mortality rate in patients above 15 years of age with asthma[37] However, studies of long-term mortality in children and young adults with asthma are few.

1.2 THE IMMUNE SYSTEM

The main assignment for the immune system is to protect us from invading pathogens and tumours. The nonspecific or innate immune system provides a first line of defence and is not specific to a particular pathogen or antigen. The innate immune system includes monocytes, macrophages, neutrophils, basophils, mast cell precursors, the complement system and an array of enzymes.
The adaptive or specific immune system includes B- and T lymphocytes and is characterised by antigen specificity, diversity, immunological memory and self-nonself recognition. Effective adaptive immune response requires antigen presenting cells including macrophages, dendritic cells and B cells able to ingest, process and subsequently present antigen to B and T cells. Unfortunately the immune system may also react to allergens and autoantigens leading to development of allergic and autoimmune diseases[38].

1.2.1 B lymphocytes

B cells are responsible for the humoral immune response. Naive B cells recognize antigen by membrane-bound antibody molecules. When encountering a matching antigen the B cell becomes activated and differentiate into memory B cells and plasma cells. The plasma cells secrete antigen-specific antibodies to the inducing antigen and have little or no antibodies bound to their cell membranes[39].

1.2.2 T lymphocytes

T cells are derived from precursors in hematopoietic tissue, especially the bone marrow. In contrast to B cells, T cells migrate to the thymus in order to mature and undergo differentiation. T cells are divided in two well-defined subgroups: T cytotoxic (Tc) cells characterized by the CD8 marker and T helper (Th) cells characterized by the CD4 marker. The latter group is further divided in two subpopulations, Th1 and Th2, distinguished by the different panels of cytokine they secret. Additional sub-populations, are Treg and Th_{17} T cells. Antibody responses to protein antigens require recognition of antigen by Th cells and co-operation between the antigen-specific B cell and Th cell[40, 41].

During the maturation process in thymus, T cells capable of binding to self-MHC are accepted (positive selection) while T cells with high-affinity receptors for self-MHC or have receptors for self-antigen presents by self-MHC are eliminated (negative selection). As a consequence only T cells that are self-MHC restricted and self tolerant will develop into mature T cells while autoreactive T cells will disappear through apoptosis[42-44].

1.2.2.1 T cell receptor (TCR) and T cell activation

T cells express a unique antigen-binding molecule, the T cell receptor (TCR), on their surface. TCR specificity is subdivided into two classes either the αβ or the γδ and their corresponding T cell subset named αβ and the γδ T cells. T cells with the αβ subset are further divided according to their co-receptors $CD4^+$ (Th) or $CD8^+$ (Tc).

The cytoplastic (C-domain) of the TCR is short and unlikely to participate in signal-transduction to intracellular components. Instead the transmembrane part of the TCR domain induces T cell activation through the TCR-CD3-complex[45, 46].

The αβ T cell receptor recognises only antigens bound to, and presented by antigen presenting cells (APC) associated with MHC-molecules. However, in contrast to αβ T cells, the γδ T cells do not require either MHC processing or presentation for antigen recognition in a manner which seems more consistent with innate immunity. T cells use the TCR to scan APC´s for processed cognate antigenic peptides presented by MHC molecule (pMHC)[45]. Upon recognition of cognate antigens the T cell forms an **immunological synapse (IS)**[47, 48] for contact with APC. Except the TCR/pMHC pair, major components forming the IS are CD28, CD4, CD8, interleukins and LFA-1.
To become fully activated, and not anergic (unresponsive), T cells require additional **co-stimulation** from CD28, often referred as the "secondary signal". CD28, expressed on almost all resting CD4+ T cells and 50-80% of all CD8+ T cells, has two ligands, B7-1 (CD80) and B7-2 (CD86)[49]. Both these ligands are constitutively expressed on dendritic cells and induced on activated macrophages and activated B cells. T cell activation through the IS induces T cell proliferation and cytokine production.

1.2.2.2 Cytokines

Cytokines are soluble regulatory molecules mediating communication between cells. When cytokines are secreted by, and acts on other leukocytes they are also referred to as interleukins. Major producers of cytokines are Th cells, denditric cells and macrophages. Cytokines bind to specific receptors on the surface membrane of target cells and regulate intensity and duration of e.g. immune responses (Table 1).

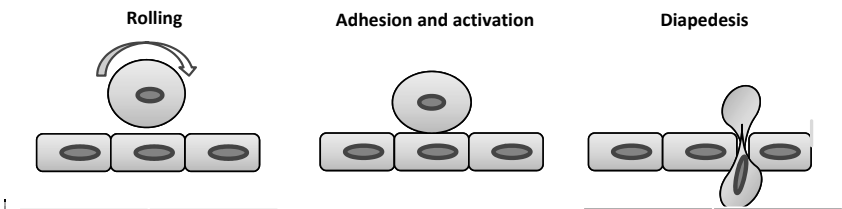
Interleukin-2 (IL-2) and interleukin 4 (IL-4) are of special interest in the context of autoimmune and allergic diseases[50-52]. After many years of assuming that IL-2 was solely a growth factor for T cells it is now clear that that this interleukin is essential for down-regulation of immune response through induction and maintaining of Treg cells[51, 52]. As a result IL-2 is used in the treatment in autoimmune diseases and GVH-reactions[51, 53]. In contrast IL-4 is associated with adverse responses promoting allergy and autoimmunity.

Cytokine	Source	Functions
IL-2	TH1	T- and B cell proliferation, cytokine release, differentiation of CD4 cells
IFN-γ	"	Macrophage activation, enhance antigen presenting by dendritic cells
IL-12	"	TH1 differentiation
IL-3	TH2	Induce eosinophil proliferation and mast cell degranulation
IL-4	"	TH2 differentiation, B cell maturation and switch to IgE production
IL-5	"	Eosinophil activation
IL-10	Broad	Major suppressive cytokine
TGF-β	Broad	Wound repair, Treg maintenance, inhibits activation of T cells and macrophages

Table 1. Examples of major cytokines produced by T cells

Chemokines, a group of cytokines plays an essential role in regulating T cell motility as well as most others leukocytes[54]. A subgroup, "inflammatory chemokines" control

1.2.1 T lymphocyte adhesion and motility



T cell	Endothelial cell
L-selectin	GlyCAM-1 CD34

T cell	Endothelial cell
LFA-1	ICAM
CCR7 CXCR4	CCL19 CCL21 CXCL12

T cell	Endothelial cell
LFA-1	ICAM
CCR1 CCR3 CCR5	CCL5 (RANTES)

Figure 1. Major cell adhesion molecules and chemokines involved in lymphocyte extravasation.

Lymphocytes and particularly T cells have a unique capacity to reposition themselves between the vascular system and lymphoid and non-lymphoid organs[57]. The process of continual lymphocyte recirculation is essential to allow a maximal number of antigenically committed T cell**s** to encounter their cognate antigen. T-cells exit blood vessel**s** through a multistep process comprising rolling, adhesion, activation and finally diapedesis through junctions between endothelial cells as depicted in Figure 1**.**

Lymphocytes circulating in the blood have a round shape while adhesion, extravasation and migration in tissue T cells require a dynamic and flexible morphology. Accordingly, the migrating T cell show an elliptoid, "amoeboid" cell shape with a pseudopod protrusion at the leading edge and a trailing uropod[58]. Antigenic challenge induces a proliferative response by arresting migration of T cells whereas tolerance maintains migration[59-61] .

Naive T cells extravasate only trough regions with specialized endothelial cells in postcapillary venules "high-endothelial venules" (HEV) localized in most secondary lymphoid organs[62]. Rolling is mediated by L-selectin (CD62L) expressed on the T cell binding to GLY-CAM or CD34 on the HEVs[62, 63]. In the next step chemokines (CCL 19 and CCL 21) on endothelial cells binds to their receptor CCR7 on the T cell mediating activation of integrins LFA-1 and $\alpha 4\beta 1$[56]. These two integrins expressed on

naive T cell binds to their ligands VCAM-1 and ICAM-1 on the endothelial cell resulting in firm adhesion[64].

In contrast to naive T cells, activated T cells may also extravasate through the endothelial tissue outside lymphoid organs. This extravasation is not random as it has a tendency to occur in localized area or in specified organs. As a result effector T cells tend to home to regions of infection and memory cells to the type of tissue in which they first encountered antigen. This "homing" process is facilitated–in that the relevant subset of T cells lack the expression of CD62L and CCR7 and instead their migration is directed by tissue specific integrins and chemokines resulting in homing to e.g. the Payer´s patch, small intestine, skin, nasal-associated lymphoid tissue through MALT and lung (BALT). Increasing evidence indicate that antigen presentation by the endothelial cell contributes to the development and specificity of T cell adhesion and extravasation[65-67].

At sites of localized inflammation cytokines induce an up-regulation of adhesion molecules on endothelial cells such as E- selectin, P-selectin, ICAM and VCAM together with increased expression of chemokines such as CCL2 and CCL17. These molecules interact with LFA-1, L-selectin and chemokine receptors (e.g. CCR2 and CXCR4) resulting in adhesion and extravasation. The complexity of these mechanisms is shown in a murine model demonstrating that T cells are activated in the lung before entering their target tissue and inducing autoimmune disease[68-71].

1.2.2 Regulation of the T cell immune response and tolerance

A first line of protection from autoimmune diseases is mediated through **central tolerance**, the process when autoreactive B cells are eliminated in the bonemarrow and autoreactive T cells in thymus. However, some autoreactive B and T cells escape this central deletion and appear in the periphery. To prevent these autoreactive cells from causing autoimmune diseases they are normally inactivated by **peripheral tolerance** mechanisms **and peripheral** T cell tolerance to environmental antigens is crucial for avoidance of allergy[72, 73].

Important components involved in regulation of T cell responses and induction of peripheral tolerance are mechanisms regulating T cell migration and the ability to stably conjugate with APC. As mentioned above, antigen challenge in vivo inhibit the motile behavior of T cells from a random walk to arrested migration, contact with and swarming in the proximity of APC cells inducing a proliferative response [59-61] Two co-stimulatory molecules of the CD28 family, CTLA-4 and PD-1 promote peripheral tolerance by attenuating or terminating an ongoing T-cell response[74, 75].

CTLA-4, only expressed on activated T cells, restricts autocrine IL-2 production and inhibits cell cycle progression. At least to some extent CTLA-4 acts by blocking the CD28 ligands (B7-1 and B7-2) and thus preventing the effects of CD28 implicating an additional pathway of "fine tuning" T cell activation[76]. The negative regulatory molecule PD-1 is, despite its name, "programmed cell death" associated with

downmodulation rather than apoptosis. PD-1 is, compared to CTLA-4, more implicated in peripheral tolerance and accordingly protects from the induction and maintenance of autoimmune processes in the periphery[74]. Although involved in peripheral tolerance, CTLA-4 is the primary negative stimulatory molecule within the secondary lymphoid tissue[75, 77].

Both CTLA-4 and PD-1 overrides the TCR-induced arrest of migration which implies that the T cells continue to move as if they never encountered antigen[74]. Inhibiting the "arrested migration" may thus result in immunological tolerance and protection against autoimmune diseases. Accordingly, mutations in the CTLA-4 gene have associated with several autoimmune disorders such as autoimmune hypothyreoidism and type 1 diabetes and administration of CTLA-4 is reported to induce transplantation tolerance[75, 77-80]. PD-1-defient mice are shown to develop autoimmune diseases such as type 1 diabetes and arthritis[75]. Although signals generated by TCR and co-stimulatory molecules are required for optimal T cell activation and induction of tolerance it is still largely unknown how these signals are integrated by the cell.

1.2.2.1 T-reg cells

- Prevention of autoimmune diseases by establishing self-tolerance
- Suppression of allergy and asthma
- Induction of tolerance against dietary antigens
- Induction of maternal tolerance to the fetus
- Suppression of pathogen-induced immunopathological reactions
- Regulation of the effector class of the immune response
- Suppression of T cell activation triggered by weak stimuli
- Feedback control of the magnitude of the immune response by effector Th cells
- Protection of commensal bacteria from elimination by the immune system

Table 2. Functions proposed for Treg cells (modified after A Corthay[81])

A T cell population that could suppress immune responses was described in the early 1970s. This subset, shown to be $CD4^+$ T cells co-expressing CD25, is called T regulatory (Treg) cells and defined as T cells in charge of suppressing potentially deleterious activities of Th cells. Impaired Treg cell development and function is associated with autoimmune disorders and allergy[82, 83] (Table 2). However, the classifications and exactly characteristics of Treg cell remains controversial[81]. In addition to CD25, a generally accepted marker of Treg cells is Foxp3, a transcription factor which is believed to control many of its function[84]. This definition of T-reg cells has been questioned as both CD25, Foxp3 and several others markers of Treg are expressed on activated Th cells[85, 86]. Consequently, a generally accepted Treg-specific marker is still lacking. Additionally, the ability to suppress T cells is not an exclusive property of Treg cells as all $CD4^+$ T cells appear to exert various kinds of suppressive activities. For instance, Th1 cells secrete IFN-γ slowing down the proliferation of Th2 cells. In contrast, the secretion of IL4 and IL-10 by Th2 cells hamper Th1 development.

Suppression mediated by Treg cells is antigen specific. Since the discovery, Treg cells have been suggested to be involved in several immunomodulating reactions of which some are listed in table 2. Transforming growth factor β (TGF-β) protects against autoimmunity. TGF-β and IL-10 has been suggested to be a mediator of Treg mediated suppression. Several different mechanisms have been proposed to explain how Treg cells discriminate between cells to suppress and cells not to react with. So far no single model explaining this diversity has been generally accepted[87].

Whilst several studies identify the capacity of Treg cells to control allergic airway inflammation there is no general agreement on the mechanisms involved[88-91]. In experiments with sensitized mice systemic administration of purified antigen-specific CD4+CD25+ Treg cells before challenge inhibited BHR, eosinophil recruitment while effect on Th2 cytikines are controversial. In contrast, depletion of CD25+ cells before allergen challenge resulted in significantly increased Th2 cytokine response, IgE levels, eosinophilia and BHR[51, 92-96]. Kearely and co-workers have been able to show that therapeutic transfer of CD4+CD25+ Treg cells not only resolve established allergen-induced pulmonary inflammation but also prevent the development of airway remodelling.[97] In vivo studies are hampered as CD4+CD25+ Treg cells mostly are obtained from peripheral blood which is obviously distal from the active site of disease[98, 99]. Additionally there are convincing evidence for a role of Foxp3+ in preventing the development of the X-linked autoimmunity-allergic dysregulation syndrome (IPEX-syndrom) affecting young boys[95].Thrombospondin-1 (TSP-1), a protein rapidly expressed in inflamed and damaged tissue induces Treg cells through ligation of its receptor CD47[100, 101]. IL-2 provides protection against autoimmune diseases through stimulation of development of Treg cells[102].
In conclusion, although Treg cells are not well characterized they represent an subset in the prevention of allergic and autoimmune diseases.

1.3 THROMBOSPONDIN-1 (TSP-1) AND LOW-DENSITY LIPOPROTEIN RECEPTOR-RELATED PROTEIN 1 (LRP1)

Thrombospondin-1 (TSP-1) belongs to a family of matricellular glycoproteins and is a trimer with a MV of 450 kDa and, on reducing gels, 175 kDa for each monomer. Each monomer consists of specific domains of which the NH_2-terminal heparin binding domain and the COOH-terminal cell-binding domain are of special importance[103-106]. The first recognized source of TSP-1 was human platelets but the molecule is today known to be expressed on a variety of cells, especially in tissues undergoing regeneration such as in rheumatoid lesions[107, 108]. Furthermore, the role of TSP-1 in inflammatory conditions is further demonstrated in animals with defective TSP-1 expression[101, 109-112]. In a model studying allergic asthma in sheep, Huang and co-workers reported already 1996 increased plasma levels of TSP after allergen exposure[113, 114]. They speculated in a correlation with inflammatory mediated platelet activation. However has since then, TSP-1 also been shown to be released from activated mastcells.

TSP-1 is furthermore an inhibitor of angiogenesis[115] and as such indirectly of tumor progression through binding to CD36 on endothelial cells in addition to regulate spreading and migration for many tumor cells.
TSP-1 interacts with T cells by binding to CD47, LRP and calreticulin and TSP-1 promotes generation of T_{reg} cells through CD47. TSP-1 is also an activator of TGF-β and may thus regulate immune response through TGF-β[100, 101, 116, 117].
Low-density lipoprotein receptor-related protein 1 (LRP1/CD91), expressed by most cell types, is a large cell-surface glycoprotein consisting of two fragments ~515 kDa and ~85 kDa respectively. As a multifunctional endocytosis receptor it mediates internalization and degradation of a large number of ligands and interacts with proteases, growth factors and matrix proteins[118-121].

1.4 ALLERGY

Among children and young adults with asthma a majority are sensitized to air-borne allergens. The term "allergen" refers to nonparasitic antigens which are capable of stimulating type I hypersensitive response in allergic individuals on repeated exposure. Allergic reactions are divided into four types. Type I-III are mediated by antibody or antigen-antibody complexes: IgE-mediated (type I), IgG- or IgM-mediated (type II), and immune-complex-mediated (type III). In contrast to type I-III the fourth type, termed delayed-type hypersensitivity reaction, is primarily T-cell mediating late reactions such as contact dermatitis against nickel or tuberculin (PPD) test.

Common features of IgE mediated allergies are associations with symptoms from the airways like asthma and rhinitis. When exposed to a relevant antigen (allergen) Th2 cells induce plasma cells to secrete IgE-antibodies. This class of anti-bodies binds to the high affinity receptor (FcεRI) on the surface of mast cells (in tissue) and basophils (in the blood) causing these cells to become "sensitised". A later exposure to the same allergen cross-links with the membrane-bound IgE on these cells causing degranulation of different pharmacological active mediators. These mediators can be divided in either primary mediators e.g. histamine or secondary mediators such as leukotrienes. The type I reaction tends to start immediate after exposure to an allergen and is usually limited to one or two organs, often involving epithelial surfaces at the site of allergen entry. However, the immediate allergic reaction is often followed by a late phase reaction involving cytokines, neutrofiles, eosinofils and T cells.
In spite of the fact that much is known about factors involved in the process of sensitization and the allergic reaction there are still several question-marks. Examples of such issues are the mechanisms responsible for different phenotype expressions of allergic diseases such as asthma and how the different factors regulating the allergic reaction interact. The role of T cells that are considered to "orchestrate" the allergic inflammation is another example of such obscurity[122].

1.4.1.1 T cell involvement in the allergic inflammation

One of the first steps in the establishment of allergic sensitization is the generation of an antigen-specific T cell with the ability to initiate response to an allergen. The main allergen presenting cell in the airways are the dendritic cells (DC) localized beneath the

respiratory epithelium. Uptake of allergens by DC is an active process which is thought to be facilitated by signals from the epithelium.[123, 124] It is speculated that these stimulatory signals are increased if the epithelium is damaged by microbes or other irritants. There are several reports indicating the involvement of Toll-like receptors (TLR), on epithelial as well as dendritic cells being involved in development of allergy[125, 126]. In the initial sensitization process the DC, when properly stimulated, will migrate to the draining lymph node where they interact with naive T cells via TCR, MHC class II and co-stimulatory molecules. Examples of co-stimulatory molecules involved in these reactions are, as described above, B7 on antigen presenting cells and CD28 on T cells. The interaction with DC is an important step in the process when naive T cells differentiate into Th1 and Th2 cells[127].

Th2 cells and their cytokines are considered to orchestrate the allergic inflammation, especially in the airways[128, 129]. The Th2 cytokines IL-5 and IL-13 is critical for allergen-induced eosinophil maturation, recruitment and survival. After the initial sensitization to an allergen IL-4 is required for the differentiation of naive T cells into Th2 cells. However, the mechanisms responsible for Th2 differentiation during the initial sensitization remain unclear. Additionally, both IL-4 and IL-13 are essential in the isotype switching of B cells to IgE synthesis. Th1 cells are also recruited into the lung during an allergic asthmatic reaction but their roles in the asthmatic response are still under debate. There are conflicting reports suggesting that Th1 cells mediate regulatory effects as well as enhanceing the allergic reaction.[130-132]

Signalling through IL-2R and CD28 increase IL-9 secretion by allergen-specific T cells which could explain why IL-9 expression increases markedly in response to allergen challenge. IL-9 has been implicated in both inflammatory and remodelling process in asthma and human trials with monoclonal antibodies against IL-9 has showed some evidence of efficacy[133].

Chemokine receptors expressed on Th2 cells, such as CCR4 and CCR8 are suggested to be responsible for the migration of these cells into the airways, however, several questions in this issue remains unanswered[134, 135].

In humans, large reservoirs of tissue-resident memory T cells (TRM) have been reported in the skin and the lungs, however it is unclear how TRM cells modulate allergic inflammation[136]. Islam et al have shown that T cell trafficking, necessary for asthmatic inflammation, involve both innate and adaptive pathways. A therapeutic approach by blocking the tissue- and inflammation-specific trafficking by T cells is the use of an antibody to CD11a (Efalizumab)[136]. It inhibits skin-homing of T cells and has been reported to be effective in some individuals with atopic-dermatitis.

2 AIMS

This thesis has two main themes. One aim was to evaluate risk factor for serious outcome of asthma in teenagers and young adults. A second aim was to characterize a mechanism regulating activation and motility of the T cell and the possible implication in allergic and autoimmune inflammation. The specific aims of this thesis were as follows:

Paper I.
To, during a 10-year period, evaluate mortality rate associated with asthma and identify risk factors for deaths in asthma in teenagers and young adults

Paper II.
To evaluate outcome of asthma, impact of atopy and other risk factors for deterioration in asthma during and after transfer from paediatric to adult healthcare

Paper III
To examine the influence of LRP1 and TSP-1 on T cell motility, adhesion and proliferation and the influence of IL-2 and IL-4 on TSP-1 expression and motility

Paper IV
To examine the influence of T cell activation by antigen on the expression of TSP-1 and LRP1 with special reference to the arrest of motility induced by activation

Paper V.
To examine T cell motility and TSP-1 expression in young adults with asthma and allergy

3 METHODS

3.1 PAPER I – ASTHMA MORTALITY

3.1.1 Study design

A national Swedish Task Force was established to monitor prospectively all deaths of children and young adults from asthma during the 10-year period 1994-2003. The expert panel included specialists in paediatric and adult asthma, respiratory medicine and forensic medicine as well as an asthma-specialist nurse.

3.1.2 Collecting information of deaths

All death certificates for 1-34 years old which in any part of the certificate contained a diagnose related to allergic or respiratory disease were received from Statistics Sweden and, later on, from the Centre for epidemiology (EPC) at The National Board of Health and Welfare. In order to identify false negative asthma deaths all death certificates with a related respiratory tract disease recorded as the underlying cause of death were collected. The specific ICD diagnostic codes used were 490-496 and 995 (ICD-9) or the ICD-10 codes J40-47 and T28. The age group 1-35 years was selected in order to avoid other pulmonary diseases such as chronic bronchitis. Children less than one year were not included because of difficulty in diagnosing asthma in this age group. Additionally, the medical profession were on several occasions asked to report all suspected deaths from asthma in the actual age group.
Police reports, medical records and autopsy reports for all individuals suspected to have died from asthma were collected. Whenever possible the asthma-specialized nurse conducted telephone interviews with the next-of-kin employing a modified standardized questionnaire developed by the British Thoracic Society and previously used in investigations similar to ours[137].

3.1.3 Analyzing of data

Information obtained concerning each suspected death from asthma was carefully reviewed by one of the member of the Task force according to a standardized protocol and subsequently presented to the rest of the panel. The Task Force met regularly to discuss about the patients and complete a final panel work-sheet in order to determine whether the patient had indeed died of asthma and, when possible, identify predisposing factors and classify the severity of asthma before death.
In addition to asthma severity specific risk-factors analyzed were; presence of allergy, under-treatment and presence of an adverse psychosocial situation.
For analysis, the subjects were divided into the following age groups: 1-19 years old (n=12, 6 males), 20-29 years old (n=12, 8 males) and 30-34 years old (n=13, 7 males).

3.2 PAPER II – ADOLESCENTS WITH ASTHMA

3.2.1 Study design

Adolescents with asthma were recruited at the time when they were transferred from paediatric to adult healthcare. Those with mild or moderate asthma assigned randomly to primary care or a specialized transition asthma clinic. Lung function, working

capacity, presence of allergy and bronchial hyperresponsiveness were evaluated when entering the study and two and five years later.

3.2.2 Study population

156 adolescents were recruited consecutively when they, due to their age, had their final visit at the Children´s Hospital at Huddinge University Hospital, Stockholm. All subjects fulfilled the ATS criteria for asthma and had visited the clinic for regular follow-up visits for at least three years. A majority of the teenagers had other allergies such as rhinoconjunctivitis, eczema and food allergies. Of the 156 patients originally recruited a total of six subjects were excluded due to mental retardation (n=2), chest-wall disease (n=1) and three declined to participate.

3.2.3 Definition of asthma severity and randomization

Mild/moderate severe asthma was defined as FEV1 >80% of predicted (% pred) managed with a total daily dose <600 µg inhaled corticosteroids (budosemid or equivalent). Those who fulfilled these criteria and had no severe food allergy were randomly assigned to the transition asthma clinic (n=51) or to primary care (n=46). The subjects who were classified as severe were all transferred to the transition asthma clinic.

3.2.4 Definition of "poor adherence"

Subjects who admitted that they on a regular basis did not take their prescribed drugs and/or missed more than two consecutive clinical visits without a reasonable explanation were classified as "poor adherence".

3.2.5 Test for allergy

Skin-prick test was performed in duplicate on the volar side of the lower arms with standard dilutions (100,000 BMU/ml) of 12 different allergens e.g., birch, timothy grass, mugwort, cat, horse, dog, house dust mite (*Dermatophagoides pternyssinus*), mold (*Cladosporium, Aspergillus fumigatus* and *Alternaria*), fish and peanut - utilizing Soluprick© allergens (ALK, Abello, Denmark). Histamine (10 mg/ml) was used as a positive and 50% glycerol as a negative control. Wheal diameters of $\geq$3 mm after 15 minutes were considered to indicate a positive reaction.

3.2.6 Lung function

Forced expiratory manoeuvres were performed according to the criteria of the American Thoracic Society (ATS)[138] in the standing position without noseclips employing a Vitalograph spirometer (Vitalograph Ltd , Buckingham, UK). All tests were conducted by one of the co-authors (K S-B) , using the same spirometer which was calibrated daily. The highest value of three valid measurements of FEV1 was recorded. Spirometric values are expressed as percentages of the reference values (% pred) provided by Zapletal[139] for individuals younger than 18 years of age and the ECCS for those older[140].

3.2.7 Bronchial challenge

Presence of bronchial hyperreactivity responsiveness (BHR) was evaluated by airway responsiveness to inhaled histamine phosphate with the method described earlier[141]. Aerosols were generated through an automatic inhalation-synchronised jet nebulizer (Spira Elektro 2) equipped to provide an adjustable aerosol delivery time. Three concentration of histamine diphosphate were used (1, 8, 64 mg/ml). At each concentration 2, 4 and 8 breath were taken with FEV1 determined 3 minutes after each dose. The dose of histamine causing a reduction in FEV1 of ≥20% (PD20FEV1) was calculated by linear interpolation on the individual log-dose response curve. Airway hyperresponsiveness was defined when the cumulative dose of histamine inducing a reduction in FEV1 was ≤880µg (PD20≤880µg).

3.2.8 Exercise test and BMI

A submaximal exercise test on an ergometer cycle (Ergomed 824E, Monark, Sweden) was performed and evaluated as according to Åstrand and Ryhmning[142-144]. Since the exercise test was primary performed to estimate working capacity and not presence of exercise induced asthma each subject inhaled a β_2-agonist and warmed up for 10 minutes prior to the test. Heart rate was measured with a Polar sport tester (PE-3000, Polar Electro OY, Kempele, Finland). The initial workload was set to 0.5 or 1.0 kP on the basis of weight, gender and exercise habits and a constant pedal speed of 50 rpm was maintained throughout the test. Heart-rate was recorded every minute until steady state was reached. Lung function was measured prior to the test and after 1, 5 and 15 minutes with a Vitalograph spirometer.
Body-mass-index was calculated as weight in kg/(height in meter)^2.

3.3 METHODS PAPER III AND IV

3.3.1 Cells

Peripheral blood lymphocytes were purified from healthy donors using Lymphoprep density gradient separation and depleted of phagocytic cells by treatment with carboxyl iron and magnetic removal. When indicated, further enrichment of T cells was accomplished by depletion of CD56-, CD19- and CD14-positive cells using magnetic beads coated with the corresponding antibodies.
The birch-specific (bet v) CD4-positive T-cell clone AF24 was obtained from XX. This T cell clone was stimulated with anti-CD3 or specific antigen. In these latter experiments with antigens autologous B-cell were used as antigen presenting cells subsequently removed by CD19 coated beads. AF24 was cultured in presence of 10 ng/ml IL-2 and, if not mentioned otherwise, in serum-free AIM-V medium (Gico Ltd., Paisly, UK).

3.3.2 Cell proliferation

Cell proliferation was determined as previously described [145].

3.3.3 Cell motility

Collagen type 1 was diluted in serum-free RPMI 1640 and H_2O (8/1/1), applied in plastic Petri-dishes 1ml/dish (30 mm; BD Biosciences) and allowed to polymerize at room temperature. A total of $1.0x10^6$ cells in AIM-V medium was added to each well and allowed to migrate for different times. Cytochalasin B, 10µg/ml prevented migration into the collagen showing that it is an active cellular process. The cells were fixed in 2.5 % glutaraldehyde or for immunocytochemistry in 2% paraformaldehyde and washed twice with PBS. Cell morphology and cell migration were evaluated in nine fixed positions in each well and at 50 µm intervals throughout the gel by the use of an inverted microscope (Nikon Eclipse TE300) and a digital depth meter (Heidenheim ND221). The results are given as mean number of infiltrating cells/field (x 20 objective) per infiltration depth (50 µm for the first two layers immediately beneath the gel surface and 100µm for other layers further down), as total number of infiltrating cells throughout the gel (x 20 objective) or as maximal infiltration depth. The infiltrating cells were identified in situ in the collagen gels using immunocytochemistry after fixation in paraformaldehyde. The transwell assay was performed using 48-well Boyden chambers. The lower wells were filled with RPMI containing 1 mg/ml BSA whereupon 8-µm nucleopore filters were placed in the chambers. The upper chamber was filled with 50 µl of $2x10^6$cells/ml in AIMV. Following incubation for 1 hour the number of cells in the lower chamber was counted in triplicate.

3.3.4 Cell adhesion

To study cell adhesion, plastic Petri dishes (90 mm. Heger A/S, Norway) were coated with ICAM-1 (2 µg/ml), fibronectin (10 µg/ml) or poly-L-lysine (10 µg/ml) and extensively washed before use. The cells (10,000/position) in AIM-V medium were incubated on the substrates in a humidified CO2 incubator at 37°C for 15 or 30 min. Cells were fixed in 2.4 % cold glutaraldehyde (GTA) for 10 min, unbound cells were removed by gentle aspiration or for identification using immunocytochemistry after fixation in 2% paraformaldehyde. The number of adherent cells per microscopic field (20 x objective) was counted. Cell adhesion was evaluated in nine fixed positions. Human plasma fibronectin and rat tendon collagen type I were purified and prepared as described elsewhere [146, 147]

3.3.5 Immunocytochemistry

The expression of different antigens was determined by quantitative immunocytochemistry of cells fixed in 2% paraformaldehyde at 4° for 20 minutes attached to glass slides. Antigen expression was detected with mAbs and a complex of biotinylated peroxidise and avidin (Vector laboratories, Burlingame, Ca). For detection of intracellular antigens cells were fixed in 2% paraformaldehyde followed by washing in buffer containing 0.1% saponin. Staining intensity was quantified using a Nikon Eclipse E1000M microscope and the image processing and analysis program ImageJ.

3.3.6 Thrombospondin mRNA expression

Messenger RNA was extracted as previously described (ref) and the RNA/protein ratio was estimated. Following PCR amplification of TSP-1 primers the product was mixed with loading buffer and separated on a 1.5% agarose gel containing 0,5 µg/ml ethidium bromide.

3.3.7 Small interfering RNA-mediated gene silencing

The expression of TSP-1 and LRP1 was suppressed using the human T-cell Nucleofector kit (Lonza, Köln, Germany) together with a Nucleafector device (Amaxa biosystems, Köln, Germany) as described by Bidere and co-workers (ref). T-cells ($5x10^6$) were resuspended in 100 µl nucleofector solution and transfected with 500 nM final concentration of small interfering RNA (siRNA) using protocol U14. The TSP-1 and LRP1 siRNA (sense and antisens are described in table xx). The resulting degree of gene silencing and the effect on motility were determined 40 hr after introducing siRNAs.

3.3.8 Biotinylation and immunoprecipitation

Biotinylation of cell surface proteins on intact lymphucytes was performed with D-biotinyl-e-aminocoproic acid-N-hydroxysuccinimide ester (biotin-7-NHS) as described by the manufactor (Roche Molecular Biochemical, Stockholm, Sweden). Adherent cells were biotinylated and released by a scraper and subsequently lysed in lysis buffer. Immunoprecititation was essentially carried out with the specific antibodies coupled to protein G agarose beads to capture the protein of interest as described by the manufacturers (Roche). Proteins were separated from the beads and antibodies followed by separation by SDS-PAGE. The proteins were transferred to a nitrocellulose Hybond ECL membrane (Amersham, Little Chalfont, UK) and detected using the BMC chemiluminescence blotting kit (Roche)

3.3.9 Western Blotting

Proteins were extracted and dissolved in lysis buffer, separated by SDS-PAGE and electrophoretically transferred to a nitrocellulose membrane. After blocking overnight the filters were incubated with antibodies. Bound antigens were visualized by chemiluminescence using ECL Western blotting reagents and Hyperfilm TM (Amersham).

3.4 METHODS PAPER V

Study population
Asthmatic patients (n=19) aged 18-26 yrs with allergic asthma were recruited from the asthma/allergic clinic at Huddinge University Hospital. All subjects fulfilled the American Thoracic Society criteria for asthma and had a skin prick test verified reaction (≥3mm) against birch pollen together with typical symptoms when exposed. Assessment of atopic diseases was carried out by a questionnaire regarding symptoms of asthma, rhinoconjunctivitis and atopic dermatitis. Peripheral blood was drawn by standard procedure. Blood samples were also collected from 14 healthy controls and 7

patients having chronic plaque psoriasis recruited from the department of dermatology at the same hospital.

Cell preparation, analysis of motility and quantitative immunocytochemistry was performed as described in methods paper III and IV.

4 RESULTS AND DISCUSSION

PAPER I,

4.1 ASTHMA MORTALITY

4.1.1 – incidence and accuracy of death certificate

During the 10-year period, 1994-2003, 75 deaths suspected to be due to asthma in individuals in the age-group 1-35 year old were reported and analyzed. In 37 of these cases deaths due to asthma were confirmed by the Task Force. As shown in figure 2 the incidence of death due to asthma in this age-group decreased from 1.54 per million 1994 to 0.53 per million in 2003. These results are in accordance with reports from several "western" countries indicating a break in the trend of increasing mortality due to asthma in this age group[35, 36]. This decline is thought to be due to improvement in management of asthma, especially the introduction of inhaled corticosteroids and improved guidelines for treatment of asthma[35, 36].

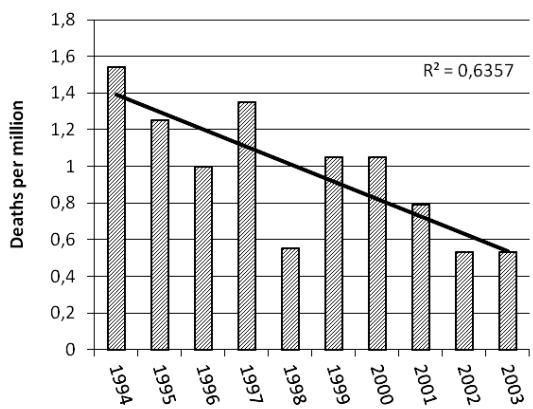


Figure 2. The incidence of deaths due to asthma among 1-34 years old in Sweden from 1994 to 2003 as determined by the Task Force

When analyzing studies of mortality from asthma it is important to know how accurately death certificates identify asthma deaths. Death certificate may report a disease as being either the underlying or a contributory cause of death which may have consequences as official statistics reflecting primarily the reported underlying cause[34]. In 46 cases asthma was recorded as the primary cause of death in the death certificate of which the expert panel disagreed about 14, indicating an over-estimation of 30%, Figure 2. Of the 37 deaths classified by the expert panel as being due to asthma this diagnose was not regarded as the primary cause of death in five cases indicating under-estimation

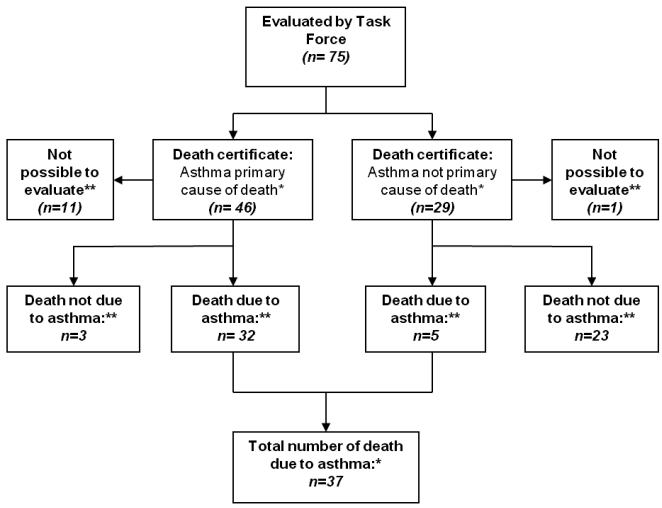


Figure 3. Comparison of the cause of death according to the death certificate* and as evaluated by the Task Force**

(false-negative rate) of 14%. Similar figures in underestimating death from asthma are reported from Great Britain (18%) and Canada (16%)[34, 148, 149] In contrast, a Finnish study revealed a total (false positive and negative) misclassification of only 7% [150]. Possible explanations to these divergent figures are circumstances such as methods used to estimate the validity of death certificates, investigated age span and criteria for defining "true asthma death". In our study autopsy was performed in 76% of cases but the usefulness of post mortem findings has been questioned in that microscopic as well as macroscopic findings may be uncharacteristic[148, 151, 152].
Except for two cases, all death reported directly to the Task Force by the medical profession were also documented in the death certificates indicating that most cases of suspected death due to asthma were analyzed by the expert panel. In spite of this our result, identifying 37 cases of death due to asthma, is probably an underestimation as the expert panel concluded that the information available was insufficient for confident determination of the cause of death in 12 cases.

4.1.1 Risk-factors for asthma mortality

Allergy to food and pet dander was the most prominent risk-factor associated with death by asthma in 19 subjects of whom only two belonged to the group of 30-34 years old adults, figure 4. An allergic reaction to food, especially allergy to soy and peanuts, was the major cause of death in the 1-19 years old individuals which is in line with previous reports[31, 153, 154]. It is notable that a majority in this group was considered to have mild or moderate asthma which, to some extent, may be explained in that half of the subjects in this age group were on inhaled corticosteroids, figure 4. However, unexpected death in children with mild disease has been previously reported before inhaled corticosteroids was a general accepted regime in treating children with asthma[31, 155]. Our results are in accordance

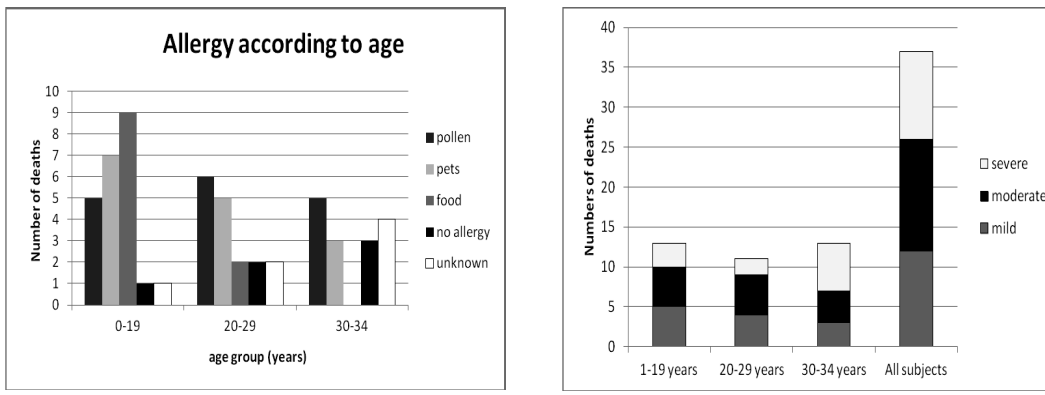


Figure 4. Distribution of deaths due to asthma according to age, allergy and severity.

with previous reports that almost all death caused by food anaphylaxis are due to anoxaemia caused by asthma[31, 153, 154]. The need of distinguishing anaphylaxis from acute severe asthma has been discussed as a predominance of respiratory symptoms in a patient having an anaphylactic reaction may be misdiagnosed as solely acute asphyxic asthma and subsequently result in undertreatment of the acute attack[155]. Our study revealed that in eight subjects, with a known allergy to pet dander, death was associated with exposure to this specific allergen which is a risk-factor previously seldom reported. In the majority of deaths caused by an allergic reaction the deceased were aware of their allergy but did neither avoid the specific item nor did they bring with them an auto-injector with adrenalin.

Similar to what has been reported in several previous studies[33] a great part, 17 out of 34 patients for whom this could be assessed, had deteriorated in their asthma several hours or even days prior to their final attack. With only a few exceptions this group with increasing symptoms had delayed in seeking medical help.

Under-treatment (n=23) was most evident in the groups of 20-29 and 30-34 years old. In 16 subjects under-treatment was due to the patients being non-adherent, in another six cases due to medical profession underestimating the severity and both for one individual. A possible link between asthma mortality and the use of long-acting β2-agonists has been discussed; however in our material we found no such association[156, 157].

Only two of 37 subjects who died due to asthma had not been previously diagnosed as asthmatic which is in line with a similar study from Denmark suggesting that a major part are known by the health care system prior to their final asthma attack[33]. Reliable lung function tests were available in a minority of cases in our material. However the value of such measurements in predicting asthma death is not established[158]. Becker et al could not show, in a newly presented study in adults, any correlation between BHR or FEV1 and mortality in asthmatics[158].

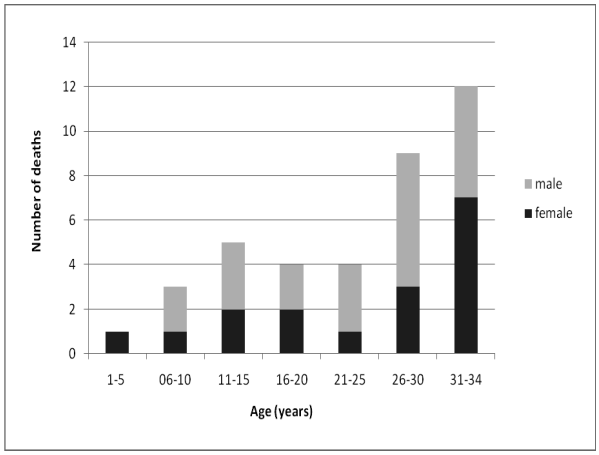


Figure 5. Distribution of deaths due to asthma according to age and sex.

As a majority of those who died due to allergic reaction had mild or moderately severe asthma, unawareness of the serious consequences of ignoring their disease could be a possible explanation. A relationship between poor perceptual accuracy of asthma symptoms and higher asthma morbidity and mortality has been shown[159-161]. The ability by the profession to improve the patient's perceptual accuracy may ameliorate the quality of self-management plans[160]. In this context, an exercise test with dyspnoea scores has been suggested as a method to identify patients with a decreased perception of dyspnoea[162]. Altogether there are several circumstances indicating, in addition to optimal pharmacological treatment, a potential role of adequate patient/family education and individual management-plans in preventing mortality due to asthma[33, 34, 36].

In contrast to previous studies[33] no seasonal variation in the frequency of mortality due to asthma was detected in our study, irrespectively of the presences or absence of pollen allergy. The limited numbers of subject may explain this diversity.

Drug and/alcohol abuse (n=8) and especially psychosocial problems (n=19) contributed to the death in the 30-34 year old adults pointing to the importance of identifying this group. The need and benefit of intervention in this subgroup of asthma patients is emphasized by reports that special clinics for such patients is shown to reduce asthma mortality[163-165]. Intravenous intake of narcotic drugs immediately before the fatal attack was found in 6 subjects. Unexpectedly, five of these deaths were associated with amphetamine which, in contrast to deaths related to inhalation of heroin, has seldom been reported[166].

4.2 PAPER II

ADOLESCENTS WITH ASTHMA

4.2.1 Risk-factors for asthma deterioration during transition from pediatric to adult healthcare

As mentioned in the method section, factors analyzed as prognostic parameter were gender, poor adherence, exposure to tobacco smoke, pets at home, BMI, regular exercise, positive prick-test, treatment with inhaled corticosteroid or transfer to primary care. Neither lung function, working capacity nor BMI was affected by any of these parameters.
Poor adherence to treatment was the only parameter with a negative impact on BHR at the five-year follow-up (OR 4.38) while regular physical activity had a positive impact on the same parameter at this time point (OR 0.34). A positive skin-prick test to furry animals and female gender had a negative impact on BHR at the time of entry into the study, Table 3. These results are further presented and discussed below.

	At entrance	*2-year follow up*	*5-year follow up*	*All three obs pooled*
Pos skin prick test	*2.39(0.28-20.66) p=0.43*	***10,9(1.30-91.70) p=0.028***	*10.07(0,95-106.9) p=0,06*	***5.10(1.19–21.70) p=0,03***
" " " " furred animal	***4.88(1.62-14.65) p<0,01***	*2.86(0.88-9.28) p=0.08*	*0,32(0.07-1.46) p=0,14*	*1.76(0.83-3.74) p=0,14*
Pets at home*	*1.57(0.58-4.23) p=0.37*	*2.00(0.69-5.7) p=0.20*	*0.99(0.33-2.84)p=1,00*	*1.14(0.71-2.78) P=0,32*
Inhaled steroids*	*2.39(0.87-6.59) p=0.09*	*1.05(0.36-3.04)p=0.90*	*2.08(0.60-7.12) p=0,25*	*1.70(0.88-3.30) p=0,12*
Female sex	***3.16(1.15-8.68) p=0,03***	***3.90(1.42-10.72) p=0.008***	*1.84(0.72-4.66) p=0,20*	***2.46(1.35-4.50) P<0,01***
Randomization prim care	*0.58(0.15-2.17) p=0.42*	***0.98(0.27-3.45) p=0.97***	*0.36(0.10-1.32) P=0,12*	***0.61(0.26-1.44) p=0,26***
Poor adherence†	*4.00(0.82-19.33) p=0.09*	***8.33(1.69-43.09) p=0.01***	***4.38(1.17-16.3) p=0,03***	***4.22(1.36-13.14) p=0,01***
Regular physical activity*	*0.5(0.19-1.36) p=0.17*	*1.31(0.47-3.66) p=0.61*	***0.34(0.12-1,00)p=0,05***	*0.63(0.34-1.19)p=0,16*
BMI > 25*	*0.32(0.08-1.28) p=0.11*	*2.03(0.53-7.77) p=0.30*	*0.76(0.22-2.57) p=0,66*	*0,90(0.41-1.97) p=0,78*

*at entrance. †poor adherence to asthma treatment

Table 3. The odds ratio (OR) obtained from logistic regression analysis of PD_{20}FEV1 histamine < 880 µg. Statistically significant values are indicated in bold. The parameter values at the time of entry into the study were used as the independent variables,

4.2.2 Atopy

At entrance of the study, skin prick test revealed that among adolescents with asthma 89% were sensitized towards at least one of tested allergens without any major changes during the course of the study. These figures had a clinical relevance with an association between symptoms and sensitization in 76.4% of those demonstrating a positive reaction to perennial allergens and in 82.5% in the case of pollen allergens. At entrance of the study 28% demonstrated a positive skin-prick test to peanuts in some cases probably due to cross-reactivity to birch[167]. The high frequency of sensitization to pollen and furry animal among ours patients is in agreement with previous studies of similar groups of young people with asthma and does not reflecting incidence in the general population[25, 26, 168]. However, sensitization in the late adolescent period has been proposed to mediate a negative impact on the outcome of asthma in later adulthood[4, 26].

4.2.3 Lung function and bronchial challenge

At entrance of our study 11% presented with a FEV1 < 80% of predicted of whom the majority improved while FEV1 deteriorated in 4 out of 104 subjects. No correlation was found between FEV1 or any tested risk-factors. As the majority of patients with FEV1 < 80% improved our result does not support previous reports suggesting a poor outcome in this subgroup of asthmatics[25, 30, 169]. Furthermore these results indicate that lung function is an insensitive indicator of asthma severity in the adolescent period an assumption in line with studies on younger teenagers[170]. However, we did not perform any bronchodilator reversibility test which might improve the benefit of lung function test.

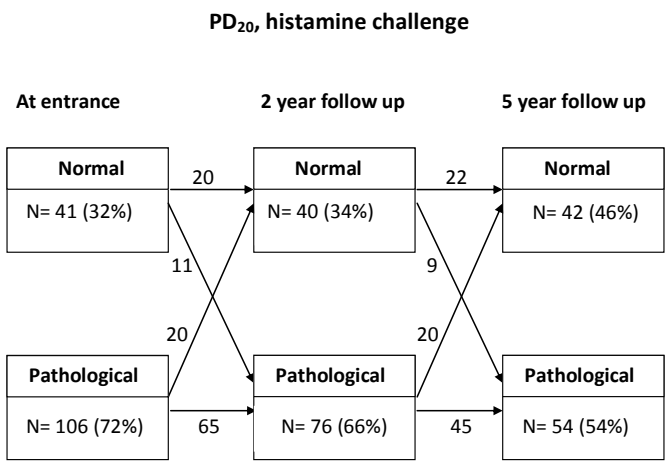


Figure 6. Normal and pathological results in PD_{20} histamine at entrance and follow up. Pathological result: ≤ 880 µg.

Bronchial challenge with histamine showed that 71% had a bronchial hyperreactivity (PD20≤880 µg) at entrance, Figure 6. Although the proportion of patients with BHR decreased during the study a considerable number deteriorated during the same period, Figure 7. This fluctuation in BHR may indicate that this subgroup still has "latent" asthma with an associated elevated risk for relapse as has been discussed previously[24, 26, 27].

Although BHR, PEF variability and bronchodilator reversibility are important characteristics of asthma these markers cannot be used interchangeably, especially not in epidemiological studies as stressed by Ulrik et al[171]. This group quoted that BHR and PEF variability were significantly associated with FEV1 whereas bronchodilator reversibility was associated with FEV1/FVC ratio and subsequently they advocated bronchial challenge tests.

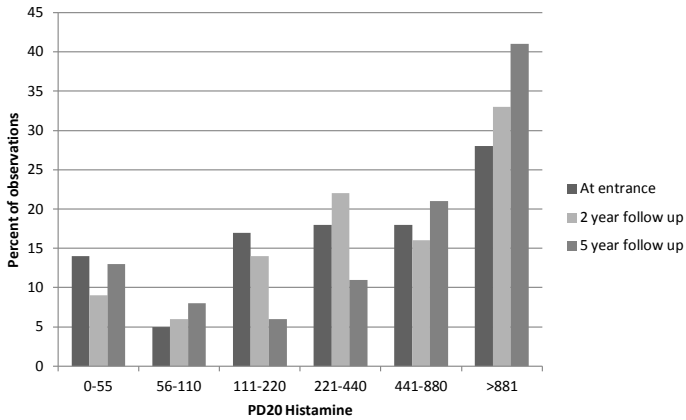


Figure 7. Bronchial responsiveness to histamine at the time of entry and in connection with the two- and five-year follow-ups. $PD_{20}FEV1$ = the dose of histamine that caused a 20% reduction in FEV1

4.2.4 Exercise test and physical activity

Working capacity and BMI was evaluated in 123 subjects at entrance and repeated by 91 of those in connection with the five-year follow-up. Although maximal exercise testing is considered the gold standard for assessing maximal aerobic capacity, the role of such testing is often limited in people whose performance is impaired by bronchial obstruction or fatigue rather than exertion[143]. Furthermore when a maximal exercise test is performed without achieving testing criteria such as age-predicted heart rate (e.g. due to muscular fatigue) the results are usually difficult to interpret[172]. As a consequence we used a submaximal exercise test ("Åstrand and Ryhmning (A-R) Cycle ergometer Test") as described in "Methods" [142-144]. This test is based on the linear relationship between heart rate and oxygen consumption and has a high correlation with maximum oxygen consumtion[173-176].

As shown in Figure 8 working capacity decreased significantly during the study period both if measured as total oxygen uptake or oxygen uptake in relation to weight the latter could be of importance as the BMI increased during the same period (se below). No significant correlation between the performed oxygen uptake and any of tested risk-factors examined was observed. Participants in regular physical declined during the same period which we consider as the most likely explanation for the decreased working capacity. The exercise test was performed to estimate working capacity, and not to confirm exercise induced asthma, subjects were premedicated with a β2-agonist, presence of exercise asthma could not be excluded. However, only two subjects performed with a decrease in FEV1 of >15% during the test.

Physical activity levels are known to be falling among teenagers and young adults in most industrialised nations[177-181] although the number of studies addressing a possible association with asthma in this age group is limited. However, our result indicates that, properly treated, presence of asthma would not prevent patients from participating in physical activities. Furthermore, when entering our study the majority (55.4%) exhibited high or very high working capacity which is inconsistent with their asthma being a limiting factor.

This conclusion is in coherence with previous studies investigating children and adults with asthma[67, 182, 183]. In spite of this a majority of young people with asthma is reported to believe that their physical activity is reduced as a result of their disease[184].

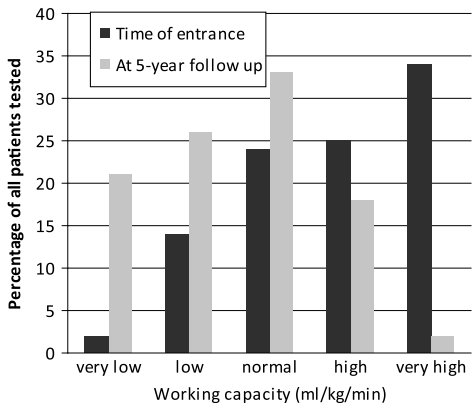


Figure 8. Assessment of working capacity (oxygen uptake) at the time of entryand the five-year follow-up. The data were categorized according to Åstrand.

A possible explanation for these contradictory observations could be a vicious cycle of decreased physical activity leading to deterioration of fitness during the transition from being a teenager to becoming a young adult. This decline could also easily be misinterpreted as exercised asthma by the patient as well as by health care providers. However, our finding that regular exercise was associated with less pronounced BHR at the five year follow up ($p=0.004$) may be an indicator that suffering from asthma exerts a negative effect on both the motivation for and possibility of performing physical activity, as discussed by Aaron et al[185]. An alternative explanation could be that regular exercise actually ameliorates BHR a matter that has been widely discussed[180, 182]. Of possible importance in this context is a study indicating that among young women regular physical activity increases heath-related quality of life[186]. Participating in sport activities has a documented effect on physical fitness and quality of life in children and adolescents[187]. Newly diagnosed adolescents with asthma have been shown to be less fit with lower physical activities as compared with healthy controls but improving after anti-inflammatory asthma treatment[188-190].

4.2.5 BMI

When investigating influence of BMI we found no associations with any of the tested dependent variables, although the mean value for BMI increased from 22.4 to 23.8 (SD3.8) during the study period. The numbers of patients with BMI>25 increased from 24 (19%) to 32 (35%) at the same period, possibly the result of a sedentary life style as discussed earlier. Association between asthma and overweight is a matter of discussion[191-193]. However, in our study the increase of BMI did not correlate with results from histamine bronchial challenge.

4.2.6 Adherence to treatment and consequences of transition to adult health care

In our study, 24% of those who participated in the entire five –year study (n=104) were characterized as demonstrating "poor adherence" to asthma treatment in despite of several efforts to overcome issues such as forgetfulness and ignorance. This subgroup of patients had a significantly lower logPD20 and an increased risk for BHR in comparison to those with better compliance emphasizing the need of some sort of intervention. As the group of those with poor adherence exhibited no further deterioration during the study this suggests that the increased BHR in this subgroup was due to long-term undertreatment. In the light of increased denial of their asthma among males, mentioned above, we could not show any relation to gender when analyzing those with poor adherence. A typical manifestation of poor adherence is non-attendance to scheduled appointments, which is reported to be higher than average in asthma clinics[10, 194-196]. Telephone reminders have been shown to improve attendance[196, 197]and may be replaced by new media such as SMS-messages[198].

4.3 PAPER III

A cytokine-controlled mechanism for integrated regulation of lymphocyte motility, adhesion and activation

A major function of T cells is to scan APC´s for antigenic peptides in the context of self-MHC throughout the body whereupon recognition of cognate antigens arrests motility and initiates specific proliferative responses[49, 199]. To carry out this function T cells continuously recirculate between blood and tissues through adhesion to endothelial cells accompanied by diapedesis hereafter the cells migrate in lymphoid and non-lymphoid tissues[200, 201]. In contrast, induction of antigen-specific tolerance prevents the arrest of motility. Therefore, the coordination of T cell motility, adhesive interactions and proliferation most likely plays a pivotal role for regulation of immune responses and tolerance. However, very little is known about this coordination. Another important issue, which remains poorly understood, concerns how the vital T cell functions motility, adhesion and proliferation are influenced by external stimuli via cytokine receptors.

The responsiveness of cells to external stimuli, such as integrin ligands and cytokines, is generally assumed to reflect signalling from a preformed landscape of surface receptors to intracellular networks. However, this concept does not explain how different stimuli are integrated by the cell. We examined the possibility that communication between cell surface receptors may be part of a dynamic cell-intrinsic regulation of T cell function with special reference to motility and adhesion.

To study the influence of TSP-1 and LRP1 on cell-shape, adhesion and motility we used SiRNA-mediated gene silencing of these proteins in blood T cells. **Transfection with LRP1 SiRNA** induced a switch in cell-shape from a polarized motile to an apolar rounded morphology and inhibited migration into a 3D collagen gel accompanied by increased adhesion to ICAM-1. T cells **transfected with TSP-1 SiRNA** showed a a polarized and elongated motile cell shape together with impaired adhesion to ICAM. Silencing of TSP-1 had an inhibitory effect on T cell migration although not as strong as LRP1 SiRNA (Figure 1, paper III). These findings indicate that LRP1counteract adhesion and induces motility. In addition, motility obviously requires interactions with TSP-1

AG 490, an inhibitor of the JAK-STAT signalling pathway, mediated reactions similar to knocking LRP1 i.e. increased adhesion and cell surface expression of TSP-1together with decreased motility (Figure 5, paper III). These results indicate that LRP1 promotes processing of TSP-1 via JAK-signalling with accompanying effects on adhesion and motility.

To further explore a possible interaction between LRP1 and TSP-1 we used short peptides mimetic of binding sites in TSP-1; **4N1K**, a peptide mimetic of the C-terminal CD47-binding site in TSP-1 and **hep 1**, a peptide mimetic of the N-terminal calreticulin binding site of TSP-1.

4N1K increased motility and cell surface expression of both LRP1 and TSP-1. The 4N1K-induced effect on motility was abrogated by knockdown of LRP supporting that motility requires interaction between LRP1 and TSP-1 (Figure 4, paper III). Addition of pertussis-toxin, an inhibitor of the Gi protein complex, inhibited the expression of LRP1 but not TSP-1 indicating that the 4N1K/CD47 induced cell surface expression of LRP1 and TSP-1 was mediated through different pathways.

Hep 1**,** increased cell surface expression of TSP-1 and inhibited motility. A similar increase of TSP-1 was also seen after silencing LRP1 (Figure 4, paper III). As LRP1 is a co-receptor for calreticulin these results suggest that TSP-1 disappeared from the cell surface through a process involving the calreticulin-LRP1 complex while simultaneously stimulating motility. This process seemed to be associated with degradation of TSP-1 dependent on calreticulin/LRP1 (Figure 5, paper III).

Shear flow, is reported to stimulate T cell adhesion to ICAM-1[54]. We could show a possible mechanism explaining this observation in that free-floating cells exhibited a marked up-regulation of the cell surface expression of TSP-1 (Figure 5, paper III). This is in agreement with the experiment using hep-1 showing that up-regulation of TSP-1 induce T cell adhesion.

IL-2, in contrast to anti-CD3, increased synthesis and expression of TSP-1 and stimulated motility. IL-4 had no effect on TSP-1 but increased expression of LRP1 while simultaneously hampering motility.

Originally, in order to exclude knocking experiments generating negative effects on the cells, anti-CD3-induced DNA synthesis was estimated. However knockdown of TSP as well as an anti-CD47 antibody increased anti-CD3-induced DNA synthesis (Figure 6, paper III). These results points to an inverse relation between motility and proliferation which could be of importance in modulation immunological reactions in that activated T cell stop migrating and start to proliferate, a reaction stimulating inflammatory response.

In conclusion the results presented in paper III demonstrate that LRP is necessarily for T cell motility and that the motogenic LRP/TSP-1mechanism antagonizes adhesion to ICAM-1 and fibronectin as well as TCR induced proliferative responses (Figure 9 in thesis). This cascade mediates regulatory effects of IL-2 and IL-4. In addition expression of TSP-1, with known ability to protect against inflammation, was increased by IL-2.

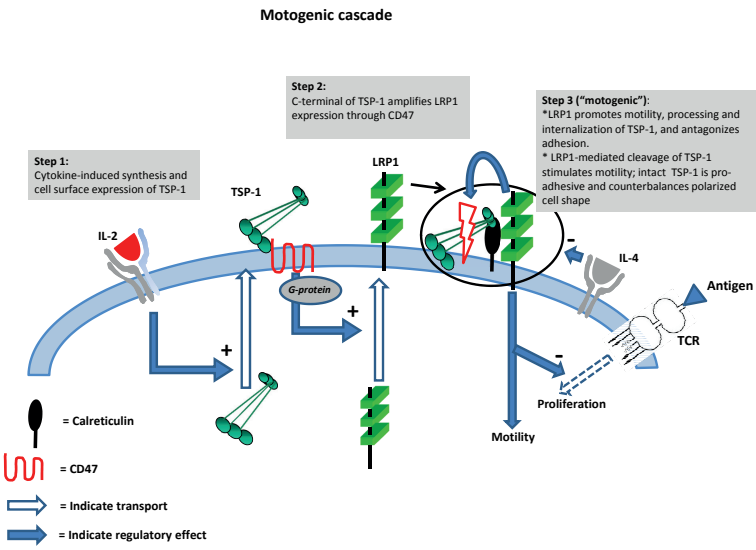


Figure 9. summarize the first part of this section and describe a cell surface cascade for integrated regulation of T cell motility, adhesion and TCR- and cytokine-induced responses. Motility is directed by cell surface-expressed LRP1 and TSP-1. LRP1 expression at the cell surface promotes polarized cell shape and migration through JAK signalling while concomitantly inhibiting adhesion to ICAM-1 and fibronectin. ERK inhibits this motogenic effect. Cell surface expression of LRP1 is enhanced by interaction of the C-terminal domain of endogenous TSP-1 with CD47, whereupon interaction of the NH-2-terminal domain with LRP1 further enhances the motogenic effect of LRP1. IL-2 stimulates synthesis and cell surface expression of TSP-1 and hence up-regulates the motogenic mechanism. IL-4 inhibits the motogenic mechanism. The motogenic mechanism inhibits TCR-induced T cell proliferation. TCR-induced proliferative responses and IL-4 inhibits the motogenic mechanism. "+" denotes stimulatory effect, "–" denotes inhibitory effect.

4.4 PAPER IV

The TCR Collaborates with CD28 to regulate T cell Motility and an Anti-Inflammatory Response through Endogenous TSP-1 and LRP1

Antigen challenge in vivo changes the motile behavior of T cells from a random walk to arrested migration, contact with and swarming in the proximity of APC cells inducing a proliferative response [59-61] . In contrast, antigen specific tolerance does not induce this arrest of migration and swarming [74]. As previously discussed the balance between T cell motility and adhesion is of great importance to maintain protection from infections and tumors without generating autoimmunity[59-61, 72, 74, 202, 203]. However, in spite of its fundamental importance for immune regulation the mechanism responsible for this altered migration by antigen is poorly understood.

We examined the possibility that antigen stimulation influenced T cell motility through the mechanism for integrated regulation of T cell motility, adhesion and proliferation dependent on LRP1 and endogenous TSP-1 as described in Paper III. Using three model systems, an allogeneic MLC, a birch allergen specific human T-cell clone (AF24) and blood T cell activated by antibodies to CD3 and CD28. Analysis of these model systems showed that an allogeneic MLC exhibited a powerful increase of synthesized TSP-1together with a simultaneous down-regulation of LRP1 synthesis and motility in contrast to T cells from the individual donors or MLC depleted of CD3 cells (Figure 1, paper IV). In the model using the AF24 T cell clone we showed a reverse relationship between motility and adhesion on fibronectin (Figure 5, paper IV)

Blocking the CD28 co-stimulating signal with abatacept virtually abolished surface expression of LRP1 and TSP-1 in MLC activated T cells (Figure 1, paper IV) and a remaining impaired motility was almost totally inhibited. To further examine the effects of co-stimulation we could show that anti-CD3 increased transport of TSP-1 out to the cell surface while CD28 increased cell surface expression of LRP1. In contrast RT-PCR revealed that anti-CD28 was permissive for synthesis of both LRP1 and TSP-1 while anti-CD3 alone as well in co-ligation with anti-CD28 almost completely suppressed mRNA synthesis of LRP1 and TSP-1 (Figure 2, paper IV).

The pronounced up-regulation of TSP-1 synthesis by antigen stimulation has several important functional implications as depicted in Figure 3 (paper IV) such as induce adhesion and macrophage activation, preventing angiogenesis and inflammation.

In conclusion, as depicted in Figure 10 (in thesis), our results indicate that the TCR induced arrest of motility reflects down-regulation of LRP1 synthesis. The concomitant up-regulation of TSP-1 synthesis may provide a mechanism for enhancement of adhesion of T cells to APC´s stimulating proliferative responses. An additional possibility is that the TCR induced TSP-1 synthesis prevents autoimmune and allergic diseases.
Furthermore, we reveal that, despite this arrest of motility, co-ligation with CD28 maintains a basal motility level by enhancing transport of LRP1 to the cell surface**.**

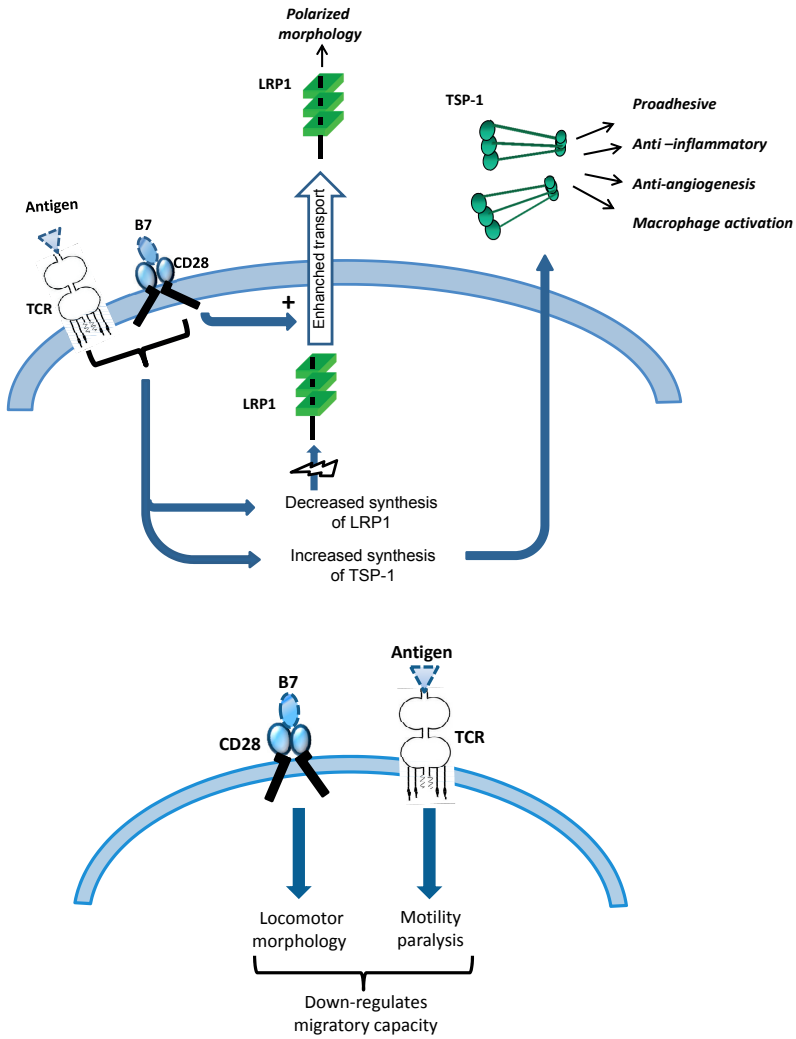


Figure 10. TCR-induced co-stimulation-dependent regulation of T cell functions through TSP-1 and LRP1. Co-stimulation of the TCR and CD28 regulates T cell motility through differential effects on LRP1 and TSP-1 synthesis and cell surface expression thus eliciting a powerful TSP-1 response/synthesis while down-regulating LRP1. Ligation of CD28 per se enhances cell surface expression of LRP1 and development of a polarized cell shape, whereas ligation of the TCR alone inhibits motility through inhibition of the expression of TSP-1 and LRP1. CD28 ligation thus counterbalances the inhibitory effect of TCR ligation alone on T cell motility. The up-regulation of the proadhesive, antiinflammatory and antiangiogenic protein TSP-1 probably serves to enhance the corresponding functions.

4.5 RESULTS PAPER V,

Dysfunctional Regulation of T cell Motility in Patients with Allergy and Autoimmunity

Many autoimmune diseases are characterized by tissue destruction mediated directly or indirectly by T cells and also involving both the humoral and innate immune system. T cells are thought to play an important role in orchestrating inflammation and infiltrate affected organs in both autoimmune and allergic diseases[204, 205].

T cells from patients with allergy and psoriasis are usually analyzed using cell surface markers defining different subsets, state of activation and proliferative capacity[134, 205, 206]. Although T cell motility play an important role in the regulation of immune responses included tolerance and effector functions motility has been little studied in these patients. However, mice with specific loss of CCR4 in their Treg cells have been shown to develop severe pulmonary disease implicating an important role for adequate T cell migration[207].
In our study T cells from teenagers and young adults with allergic asthma showed a significant reduction of migration into a 3D collagen matrices compared with both healthy controls and subjects with psoriasis. Cells from patients with psoriasis also showed impaired migratory capacity compared with controls but to a lesser extent in comparison to those with allergy (Figure 1, paper V).

According to our results presented in paper III and IV the impaired motility in patients could reflect decreased cell surface expression of TSP-1. Compared to controls, patients with allergy showed a lower cell surface expression of TSP-1 groups (Figure 3, paper V). Our results reflect an average of TSP-1 expression on T cells and not solely the allergen specific T cell subgroup possible explaining the moderate difference. As the latter subgroup of T cell is small relation to the whole cell population our results could represent a constitutional characteristic of the individuals T cell population rendering the individual more prone to suffer from autoimmune diseases. However, such a constitutional defect seems unlikely since it likely would be associated with a range of other autoimmune disorders which is not the case**.**

IL-2 was shown to up-regulate the impaired motility in patient to the same level as in controls indicating a reversible state further excluding a constitutional defect (Figure 2, paper V).
However, in the presence of IL-2 control cells exhibited an even higher level of motility compared to cells from those with an allergic disease but without any significant differences when calculated as percent of increase in migratory capacity. Another possible explanation, as suggested by our previous findings, is that the reduced motility in patients with allergy and psoriasis reflects an ongoing antigen stimulation.

5 MAIN RESULTS AND CONCLUSIONS

- A majority of teenagers and young adults with asthma are atopics and sensitized to allergens such as pets and pollen resulting in persistent exposure and subsequently high risk of having a chronic inflammation in their airways.

- Allergic reaction in form of asthma is involved in a substantial numbers of deaths due to asthma.

- Psychosocial factors such as denial and poor adherence to recommended treatment are associated with death due to asthma and increased bronchial hyperactivity indicating inflammatory activation in the airways.

- LRP1 and TSP-1 and interaction between these proteins regulate T-cell motility and adhesion.

- Interleukin-2 stimulates TSP-1 synthesis and motility and IL-2 and IL-4 interregulate through LRP1 and TSP-1.

- T cell activation induces arrest of T cell motility through down-regulation of LRP1 synthesis

- T cell activation up-regulates the synthesis of TSP-1 which may enhance T cell adhesion to antigen-presenting cells and prevent inflammation.

- CD28 regulates the cell- surface expression of LRP1.

- Patients with allergy and psoriasis showed impaired T cell motility and decreased TSP-1 expression compared to healthy controls.

6 FUTURE PERSPECTIVES

Future perspectives

Our results point out how important it is that transition from pediatric to adult health care be well prepared and planned long in advance to give teenagers the opportunity to enter adult life without being hampered by their disease. Health care must empower young people to help facilitate their own transition by becoming self-aware, fully engaged and expert on their disease.

To achieve this and to prevent deterioration in their disease, and, in the worst-case scenario, death due to asthma, we need to improve patient education, including training in the perception of serious symptoms.

The drastic deterioration in working capacity that we have observed during the transition period needs to be actively prevented. How such prevention should be optimally organized requires further studies in collaboration with experts in psychosociology and physiotherapists.

For those with a more serious disease specialized 'transition-clinics' could be of importance in achieving optimal asthma management. These clinics have to be organized and maintained in close co-operation between pediatric and adult health services.

Further studies are needed to identify those patients with risk factors associated with serious deterioration or death due to asthma.

The demonstration in the present studies of an integrated mechanism for regulation of T cell adhesion, migration and TCR-induced proliferative responses may provide a background for development of therapeutic tools to promote tolerance and interfere with adverse inflammatory responses.

Patients with asthma/allergy and psoriasis presented with decreased T cell motility and expression of TSP-1.The mechanisms behind these findings are unclear. As T cell has major role in autoimmune diseases and allergy these results may have of both diagnostic and therapeutic value.

7 POPULÄRVETENSKAPLIG SAMMANFATTNING

Astma är en av de vanligaste sjukdomarna hos tonåringar samt unga vuxna. Prevalensen (förekomsten) för astma i dessa åldersgrupper rapporteras i flertalet undersökningar ligga mellan 6-10% varav flertalet insjuknat före sex års ålder. Prognosen vid astma anses bättre ju tidigare sjukdomen debuterar. En betydande del av barn och ungdomar med astma fortsätter att ha symtom även i vuxen ålder.
Dödsfall på grund av astma hos barn, ungdomar samt unga vuxna är numer ovanliga. Under 80-talet noterades dock en oroande ökning i Sverige. I USA är astma den 6é vanligaste dödsorsaken bland barn i åldern 5-14 år. När astmasjukdomen debuterar före eller i tidiga tonår, anses förekomst av allergi spela en stor roll för den inflammation man ser vid astma.
Vid allergisk astma orsakas inflammationen i luftvägarna av kroppens immunsvar mot allergener (allergiframkallande ämnen, t ex pollen). Denna reaktion medieras av ett flertal olika celltyper varav T cellen är en.
T cellerna reglerar det adaptiva immunsvaret mot främmande antigen (**antigen** = substans som kroppens immunsystem reagerar mot, t ex bakterier) men upprätthåller normalt tolerans mot kroppens egna vävnader men även mot främmande ämnen som normalt inte är skadliga. Om denna tolerans försämras kan immunsystemet reagera mot sig själv varvid autoimmuna sjukdomstillstånd som psoriasis och reumatism kan uppträda. På samma sätt kan immunsystemet reagera mot normalt ofarliga ämnen som exempelvis björkpollen varvid en allergisk reaktion uppträder.

Arbete I. Med denna studie ville vi undersöka förekomsten av dödsfall på grund av astma i åldersgruppen 1-34 år samt försöka finna riskfaktorer för att i denna åldersgrupp avlida på grund av astma.

Under en 10-årsperiod gick vi igenom samtliga dödsbevis som angav astma eller likartad diagnos som direkt eller indirekt dödsorsak. För varje misstänkt dödsfall på grund av astma införskaffades sjukjournal, obduktionsresultat samt i förekommande fall polisrapport. Där så var möjligt genomfördes även intervju med närmast anhörig. En expertpanel bedömde om de enskilda dödsfallen var orsakade av astma samt om bakomliggande riskfaktorer förelåg.
Under perioden1994-2003 kunde vi identifiera 37 dödsfall orsakade av astma. Den årliga incidensen minskade från 1,54 till 0,53 dödsfall per million individer i aktuella åldersgruppen. Som främsta riskfaktorer för död på grund av astma konstaterades förekomst av allergi och psykosocial problematik med dålig följsamhet till ordinerad behandling men även i några fall dålig handläggning från sjukvårdens sida. Ett anmärkningsvärt fynd var att 11 av de 37 dödsfallen sannolikt orsakades av en allergisk reaktion mot föda vilket var den vanligaste orsaken bland dem under 19 års ålder. En stor del av de ungdomar som ingick i denna grupp ansågs ha en lätt till måttligt svår astmasjukdom. Åtta dödfall skedde i anslutning till exponering för pälsdjur. Bland de som avled på grund av astma i åldersgruppen 19-34 år ålder var en psykosocial problematik vanlig. Vi kunde även konstatera att dödsbevis påtagligt ofta innehöll inkorrekta uppgifter om dödsorsak.

I arbete II undersöktes under en femårsperiod ungdomar med astma i samband med överföring från barnklinik till vuxenvård. Syftet med studien var att undersöka riskfaktorer under denna period som riskerar medföra försämring av patienternas astmasjukdom. 150 ungdomar rekryterades i samband avskrivning från Barnliniken Huddinge sjukhus (numer Astrid Lindgrens barnsjukhus, Huddinge). Av de med lindrig/måttlig astma randomiserades hälften till öppen vård medan samtliga övriga deltagare följdes upp på Lung- Allergikliniken Huddinge. Samtliga deltagare genomgick upprepade undersökningar innefattande ffa allergiutredning, lungfunktion, arbetsprov o bronkialprovokation.

Som främsta resultat konstaterades att 89% av deltagarna hade ett allergiskt inslag, de behöll en god lungfunktion medan en stor andel uppvisade en kvarstående, men över tid, påtagligt varierande bronkiell hyperreaktivitet (**BHR**, överkänslighet i luftvägarna). Riskfaktor för en kvarstående BHR var dålig följsamhet till ordinerad behandling/uppföljning. Konditionen försämrades påtagligt under studietiden men utan att påverkas av undersökta riskfaktorer. Gruppen med lindrig/måttligt svår astma som randomiserades till öppen vård uppvisade likartade resultat jämfört med motsvarande grupp som följdes upp på specialistklinik.

Arbete III. Som bland annat framgår av delarbete I och II står det allergiska inslaget för en betydande del av såväl sjuklighet som dödsorsak vid astma. Det är ett välkänt faktum att T cellerna är av stor betydelse för att såväl initiera som modulera den allergiska inflammationen. I delarbete III var intentionen att studera grundläggande mekanismer som reglerar T cellernas **migration** (rörlighet), **adhesion** (att fastna) och aktivering av antigen. Specifikt har vi undersökt hur de två proteinerna thrombospondin-1 (TSP-1) samt lipoprotein receptor-related protein 1 (LRP1) påverkar T cellens **motila** (rörliga) egenskaper och hur dessa interagerar med **extracellulärmatrix** (bindväv) samt IL-2 och IL-4, signalämnen vilka anses ha stor betydelse i den allergiska inflammationen

Vi visade här att TSP-1 och LRP1 reglerar T cellens motila och adhesiva egenskaper. Resultaten visade också att interleukin-2 ökade cellens produktion av TSP-1 och motilitet, medan IL-4 hämmade motilitet. Vi har även påvisat intracellulära signalvägar som används vid de reaktioner där TSP-1 och LRP1 påverkar T cellens motila beteenden.

I arbete IV, som är en fortsättning på delarbete III, studeras de mekanismer som ligger bakom hur T cellens motila egenskaper regleras av dess specifika receptor (TCR). Vi beskriver här hur **ligering** (sammankoppling) av TCR med den co-stimulerande molekylen CD28 påverkar förekomsten av komponenter som reglerar T cellens motila samt adheranta egenskaper, framför allt TSP-1 och dess receptor LRP1. Denna reglering har även en vidare betydelse genom att TSP-1 har anti-inflammatoriska egenskaper. Våra resultat visar även att, genom antigenstimulering, aktiverade T celler leder till en stark ökning av T cellens produktion av TSP-1 samtidigt som produktionen av LRP1 och cellernas motilitet minskade. Om man i samma modell blockerade co-stimuleringen försvann såväl TSP-1 som LRP1 från cellernas yta samtidigt som deras motila egenskaper upphörde helt.

Sammantaget talar våra resultat för att TCR (T cellens receptor för olika substanser, t ex pollen) och CD28 genom att påverka T cellens motila egenskaper samt stimulera produktionen av TSP-1 kan inducera ett antiinflammatoriskt svar på stimulering.

Arbete V**.** I detta arbete undersöktes T celler från unga vuxna med verifierad allergi mot björkpollen samt från patienter med psoriasis. Specifikt studerades uttrycket av TSP-1 samt T cellernas migration in en gel av **kollagen** (liknar bindväv). T celler från patienter med allergi uppvisade signifikant lägre motilitet men ökat uttryck av TSP-1 jämfört med friska individer. T celler från patienter med psoriasis uppvisade en intermediär nedsättning av motilt beteende medan uttrycket av TSP var i nivå med allergipatienterna.

Sammanfattningsvis framkommer från denna avhandling att dödligheten på grund av astma minskat under 10-årsperioden 1994-2003. En stor del av dödfallen orsakades på grund av exponering för en faktor som vederbörande var medveten om att han/hon var allergisk mot. En annan riskfaktor var en bakomliggande psykosocial problematik. Vidare konstateras att en överväldigande majoritet av tonåringar då de lämnar barnhälsovården har någon form av allergi. Majoriteten har en bronkiell hyperreaktivitet (BHR) som framför allt kvarstår hos dem som ej skötte sin astmabehandling medan den minskade hos dem som tränade fysiskt regelbundet. Studiedeltagarnas kondition minskade påtagligt under studietiden men utan samband med någon av de undersökta riskfaktorerna. Sammantaget visar dessa resultat på vikten av en planerad överföring av ungdomar från barn till vuxenvård, behovet av patientutbildning samt förmågan från sjukvården att identifiera riskpatienter.
Det faktum att T celler från patienter med allergi och psoriasis hade en nedsatt motilitet medan IL-2, som förebygger och används vid behandling av dessa tillstånd, stimulerar motilitet kan tala för att den motilitetsstimulerande effekt som vi visat av TSP-1/LRP1 kan ha betydelse för att monitorera samt behandla autoimmuna samt allergiska sjukdomar.

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