Chapter 1, General introduction

1.1 T lymphocytes

1.1.1 T lymphocytes and the immune system

Immune responses can be subdivided into innate and adaptive responses. The innate response is immediate and not antigen-specific. It is complemented by the slower and antigen-specific adaptive response. The latter can be further divided into humoral and cell-mediated immune responses. The humoral immune system protects the organism from extracellular pathogens. The mode of action, the effector mechanism, is mediated mainly by B lymphocytes and is, therefore, antibody based. Cell-mediated responses are mainly directed against intracellular pathogens and sometimes also tumour cells. The responses are mediated by T lymphocytes and the effector function is based, among others, on cell lysis of infected or transformed cells. The initiation and regulation of an immune response involves interactions between the different lymphocytes mentioned above, as well as several other cells, making the immune system a rather complex cellular network. The cells involved are not only of haematopoietic origin, as lymphocytes, macrophages and dendritic cells (DC), but also endothelial cells in the venules or epithelial cells in the thymus (see 1.1.5) are part of the network (basic aspects of immunology are based on Paul, 2003).

T lymphocytes play a crucial role in both the humoral and cell-mediated branches of the adaptive immune response. Based on co-receptors expressed on their cell surface, T lymphocytes fall into two groups: cluster of differentiation 4-positive ($CD4^+$) and $CD8^+$ T lymphocytes, expressing the CD4 or CD8 co-receptor, respectively. This division based on cell surface markers coincides in general terms with functional division. $CD4^+$ cells mainly sustain the initiation of an immune response, the formation and propagation of immunological memory and they support humoral responses. They are, therefore, also called helper T cells. $CD8^+$ cells are cytolytic T lymphocytes (CTL). After identifying a cell as foreign, the infected or transformed cell is lysed. However, in some cases also $CD4^+$ cells can be cytolytic. The functional division of T lymphocytes can be explained by the molecular interactions of their co-receptors. The molecules bind to a complex of antigenic peptide and human leukocyte antigens (HLA) of the major histocompatibility complex (MHC) on the cell

surface of target cells. HLA molecules are expressed on the cell surface of nearly every cell and function as cellular indicators for identity and health status. The CD8 co-receptor binds to the constant region of MHC class I molecules. Class I molecules are expressed on almost all nucleated cells. This allows $CD8^+$ cells to scan all cells for possible infections and subsequently lyse them if recognised as a target. Cell lysis is mediated by Fas-Fas ligand interactions inducing apoptosis or by exocytosis of perforin and granzymes, leading to osmotic defects and apoptosis of the target cells, respectively. The CD4 co-receptor binds to the constant region of MHC class II molecules. MHC class II molecules are only expressed on specialised antigen presenting cells (APC). The prototypic APC is the DC. Also macrophages and B cells express MHC class II molecules and can present antigens to T lymphocytes. This restricted expression of MHC class II molecules allows a strict control of $CD4^+$ T cell activation. This is necessary because T helper cells are involved in the activation of CTLs, macrophages and B lymphocytes. They promote inflammation as well as immunoglobulin class switches and affinity maturation of activated B lymphocytes. T helper cells exert this function by direct cell-cell interactions and secretion of cytokines.

Upon infection of the organism with pathogens and subsequent activation, $CD4^+$ and $CD8^+$ effector cells proliferate heavily and exert effector functions. After the pathogens have been successfully eliminated, many of the effector cells die and only a few survive as memory cells. The process of memory formation is not yet entirely understood. It is clear, however, that $CD4^+$ cells play an important role in the generation and maintenance of immunological memory. The quiescent memory cells have the capability of mounting an adaptive immune response against the previously encountered pathogen more rapidly, with a greater magnitude, and more effectively compared to the primary response.

1.1.2 The T cell receptor complex and it's signalling capacity

All T lymphocytes are equipped with a T cell receptor (TCR). The TCR is expressed on the cell surface in a complex with other proteins. The TCR/CD3 complex links the antigen recognition by the TCR with the biochemical events that lead to the functional activation of T cells, which is mainly triggered by the ζ-chain. The TCR/CD3 complex

can only be expressed on the cell surface if all components are synthesised and correctly assembled in the endoplasmatic reticulum. The complex consists of a TCR heterodimer, ζ-chains and CD3 γ, δ and ε-chains. The TCR heterodimer is composed either of an α and a β-chain or a γ and a δ-chain. The α/β and γ/δ dimers are structurally very similar. The latter should not be confused with the CD3 γ and δ-chains. The two TCR chains are linked through a disulfide bond. The ζ-chains are present as a homodimer, which is also stabilised through a disulfide bond. Both CD3 δ and γ-chains form a heterodimer with the CD3 ε-chain (Pan *et al.*, 2004). Together, the two CD3 heterodimers, the ζ-chain homodimer and the TCR heterodimer compose the TCR/CD3 complex (Fig. 1A) (Call *et al.*, 2002). All proteins of the complex except for the ζ-chain contain at least one immunoglobulin (Ig)-like domain. The TCR α, β, γ and δ-chains are composed of a constant and a variable region, each region being composed of an Ig-like domain (Fig. 1B). Each T cell expresses either α/β or γ/δ TCRs. γ/δ TCR T cells form only a small subset of all T lymphocytes (less that 5%). Their precise role in the immune system is not well established. While viruses are often cleared in γ/δ knockout mice, some bacterial infections cannot be controlled, and their role in tumour immunity is under debate (reviewed in Carding and Egan, 2002). Hence, only the α/β TCR T cells will be further discussed. Nervertheless, both T cell subtypes are very similar regarding their antigen receptor structure.

T cells are stimulated through the interaction of their TCR with the cognate peptide/MHC complex (see 1.1.4) on the cell presenting the antigen. This interaction leads to the formation of an immunological synapse (reviewed in Bromley *et al.*, 2001). On both cells, on either side of the synapse, certain molecules are recruited and others excluded from the reorganised lipid rafts, which are cholesterol/sphingolipid-rich plasma membrane microdomains that sustain membrane organisation. This rearrangement creates areas of high TCR/CD3 complex or peptide/MHC concentrations, allowing serial interactions of both components. This results in triggering of signal transduction, which finally leads to the stimulation of the T cell. The signal transduction cascade is initiated by clustering of the TCR/CD3 complexes and the CD4 or CD8 co-receptors. The formation of the cluster activates the co-receptor bound protein tyrosine kinase Lck, which then phosphorylates the immunoreceptor tyrosine-based activation motifs (ITAM) on CD3

molecules and the ζ-chain. The phosphorylations lead to the recruitment and activation of the ζ-associated protein kinase (ZAP-70). Through further phosphorylation and engagement of adaptor proteins different signalling pathways are initiated. These include the Ras and Rac pathways, as well as the protein kinase C (PKC) and the calcineurin pathways. Finally, specific transcription factors as activator protein-1 (AP-1), the nuclear factor of activated T cells (NFAT) and the nuclear factor κB (NF-κB) are activated. These factors enhance the gene expression of specific target genes in antigen-stimulated T cells.

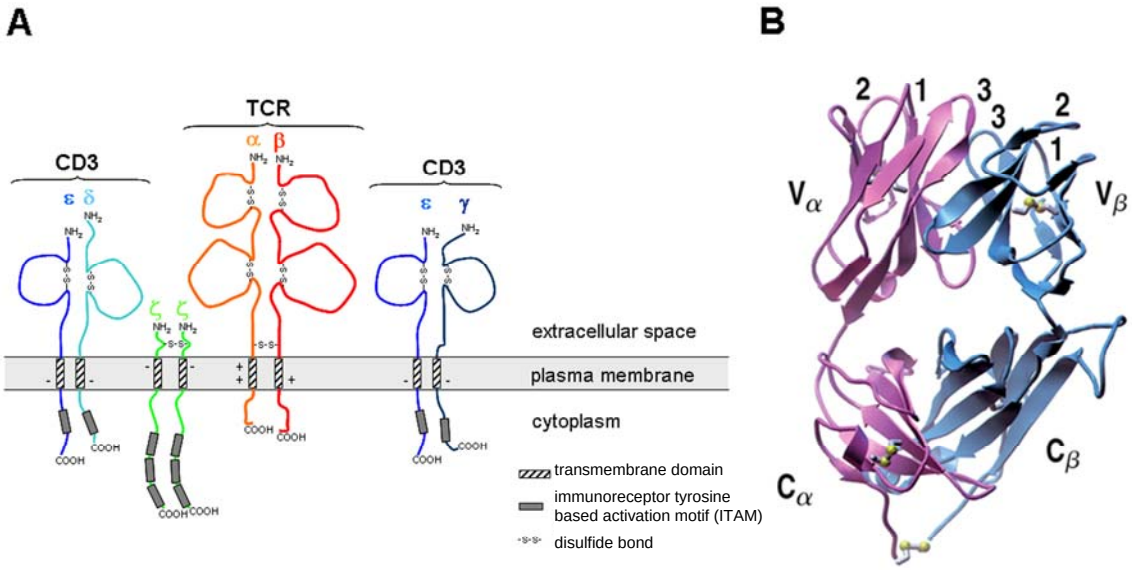

Figure 1| **The TCR/CD3 complex and the TCR structure. A)** Shown is the complex of the antigen-specific TCR heterodimer and the CD3 and ζ-chains, which are involved in signalling through their immunoreceptor tyrosine-based activation motifs (ITAMs.) The TCR/CD3 complex is stabilised through electrostatic interactions between the transmembrane domains of the proteins. The immunoglobulin-like domains are depicted as loops, which are stabilised by disulfide bonds. **B)** The crystal structure of the 2C TCR heterodimer. Clearly visible are the β-sheets and disulfide bonds of the immunoglobulin-like domains. The TCR is viewed from the side, the complementarity-determining region (CDR) loops of the antigen binding site are seen on the top (labelled 1, 2 and 3 for each chain). Figure 1B is taken from Janeway (2005).

1.1.3 T cell receptor variability

Each TCR heterodimer recognises a specific antigen in the context of a MHC molecule. In order to efficiently protect the organism from any possible pathogen, a great variety of different TCRs is needed. It has been estimated that any individual

carries 10^9 or more T cell clones of different specificity. The space needed to encode each of these TCR chains would dramatically increase the size of the genome. That is why the constant region of each TCR chain is encoded only once for the α and twice for the β-chain. By mRNA splicing the constant region and the variable region are brought together. Prior to that, the variable region is assembled by somatic recombination using one variable (V), one diversity (D, for the β-chain only), and one joining (J) segment of the same TCR chain locus (Fig. 2). The gene segments are derived from clusters of many different segments. The human TCR α locus contains 70 to 80 V segments and 61 J segments; the TCR β locus contains 52 V, 2 D and 13 J segments (Fig. 2). The random combination of the different segments leads to a large variability of TCR chains. More variability is added by random deletion or insertion of non-template encoded nucleotides in the joining regions of the different segments. The D segments of the β-chain do not contain stop codons in any reading frame. This enhances the probability of forming a functionally expressed protein when any number of nucleotides is inserted or deleted in the V-D joining region. Finally, also the juxtaposition of different randomly generated variable regions of the α and β-chain add to the variability of TCRs. The theoretical potential repertoire has been calculated to be about 10^{15} (Davis and Bjorkman, 1988). However not all recombinations of gene segments are equally likely to occur, not all TCR α and β-chains may form functional antigen receptors, and last but not least, T cells undergo functional selection in the thymus (see 1.1.5). This together leads to considerably less variability than theoretically possible: 10^8 to 10^9 instead of the theoretical 10^{15} different specificities (Arstila *et al.*, 1999).

Each T cell only expresses TCRs with a single specificity. T lymphocytes of a certain specificity are of clonal origin. During T cell development the functional expression of a TCR chain inhibits the rearrangement and transcription of the second allele (allelic exclusion) (Uematsu *et al.*, 1988; Malissen *et al.*, 1992). This tight control usually leads to the expression of TCRs with only a single specificity on each T cell. The TCR chain expression is closely linked to the tightly regulated expression of the recombination-activating genes 1 and 2 (RAG1 and 2). Furthermore, recombination is regulated by reduced accessibility to the non-recombined TCR loci, mediated by chromatin structure remodelling. In some cases however, the somatic recombination and expression of the TCR α-chain is not as tightly regulated and leads to T cells

expressing one β-chain and two α-chains (Padovan *et al.*, 1993; Heath *et al.*, 1995). Whether these T cells also gain dual specificity has not been proven. Several TCR transfer experiments have shown, however, that one of the α-chains is enough to provide the T cell with a certain specificity (our own unpublished data; Ueno *et al.*, 2002; Kuball *et al.*, 2005).

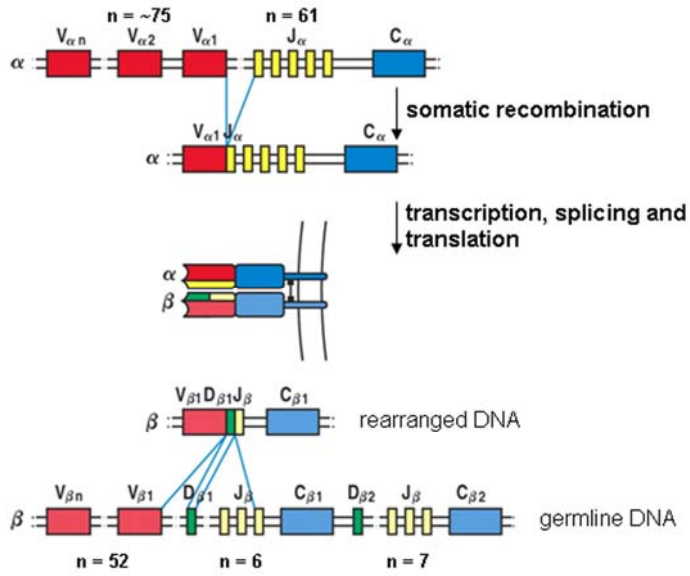

Figure 2| **The rearrangement in the TCR loci.** The TCR α and β-chain gene loci are composed of several segments that are joined by somatic recombination in order to obtain the great variability of TCR heterodimers. One variable (V) and one joining (J) region are connected through recombination. An additional diversity (D) region is included in the β-chain. The constant region (C) is only added through splicing of the mRNA. Numbers indicate the amount of the different segments. The figure is modified from Janeway (2005).

1.1.4 Antigen recognition

The TCR heterodimer is the only component of the T lymphocyte antigen receptor complex to show antigen specificity. The variable Ig-like domains of both α and β-chains endow T cells with their specificity. Both domains interact with MHC molecules and the antigenic peptide captured in its peptide-binding cleft. Within each variable domain three hypervariable regions can be detected. They are called complementarity-determining regions (CDR). The three CDR loops of each TCR chain form a flat surface that interacts with the peptide/MHC complex (Fig. 1B). Crystal structures of several TCRs indicate that the CDR 1 and 2 mainly interact with the MHC molecule, while the CDR 3 is positioned close to the peptide (Garcia *et al.*, 1996; Garboczi *et al.*, 1996). This observation is consistent with the fact that the

greatest variability is found within the CDR 3 which is the joining region of the V, D, and J segments. CDR 1 and 2 lie within the V region.

The affinity of TCRs with dissociation constants (K_d) of $10^{-4} – 10^{-7}$ is low compared to affinities found for antibodies, which lie at $10^{-7} – 10^{-10}$ (Margulies, 1997; Roost *et al.*, 1995; Foote and Eisen, 1995). This is why the half life of TCR-peptide/MHC interactions is short with only 1 to 10 seconds. This however allows a serial involvement of the TCR with peptide/MHC complexes. The weak interactions of these ternary complexes are additionally stabilised by CD4 or CD8 co-receptors. Co-receptors interact with non-variant regions of MHC molecules (see 1.1.1). This additional interaction stabilises the T cell-target cell interactions.

1.1.5 T cell maturation

"T" cells undergo maturation in the **t**hymus, as the name implies. Haematopoietic stem cells proliferate in the bone marrow and give rise to pro-T cells, which migrate to the thymus. These thymocytes neither express CD4 nor CD8 co-receptors (double-negative T cells). Also the TCR is not yet expressed. The next stage in maturation is the pre-T cell. After somatic recombination in the TCR β-chain locus, the β-chain is expressed on the cell surface. It forms a heterodimer with the pre-Tα protein and is assembled into a complex with CD3 and ζ-chain proteins. The expression of the pre-TCR is essential for survival and further proliferation of the thymocytes. In the double-positive stage, both co-receptors are expressed, and the thymocytes leave the cortex of the thymus and enter the medulla. During this stage of maturation the recombination of the TCR α-chain occurs, and the pre-Tα is substituted by the regular TCR α-chain. At this stage the T cells gain their specificity and the most important steps of T cell maturation occur: the positive and negative selection.

T lymphocytes with many different specificities arise due to random combination of different gene segments within the TCR loci. In order to function properly within the immune system, these randomly generated specificities have to be selected. First, only peptides in a MHC context are to be recognised, i.e. the TCR has to recognise self-MHC molecules. This is guaranteed after successful positive selection. The

thymic epithelial cells express MHC class I and class II molecules, which present irrelevant self-peptides. If no recognition by the TCR occurs, no survival signal is triggered and the thymocytes die of apoptosis. However, allelic exclusion for the α-chain is not as strict and rearrangement of the second α-chain can lead to a functional TCR which allows the T cell to survive. During positive selection, the development into $CD4^+$ or $CD8^+$ single positive T lymphocytes also occurs. If the TCR recognises MHC class I molecules, only the CD8 co-receptor will be further expressed. If MHC class II is recognised, $CD4^+$ cells will arise. T cells that recognise self-peptides are eliminated during the second step of selection, the negative selection. Bone marrow-derived macrophages and DCs, as well as thymic epithelial cells present a wide range of self-peptides. In case of strong recognition by a thymocyte, apoptosis is induced in the self-reactive T cell. When T cells have undergone both selection stages, functional, single-positive T cells expressing either of the co-receptors and a non-self specific TCR leave the thymic medulla and enter the peripheral lymphoid tissue. These cells have demonstrated weak binding to self-MHC during positive selection but no strong binding to self-peptide/MHC complexes during negative selection.

1.1.6 Self-Tolerance

The protection of an organism against self-targeted immune responses is called self-tolerance. Tolerance in general describes unresponsiveness to an antigen, induced by prior exposure to that antigen. Central tolerance is established in generative lymphoid organs, like the bone marrow for B cells and the thymus for T cells. Central tolerance is mainly induced by negative selection, which leads to the clonal deletion of self-reactive T and B cells by apoptosis. At peripheral sites also mature lymphocytes can be tolerised, leading to peripheral tolerance. This mechanism is important for self-antigens that are not expressed at required concentrations in the thymus. Peripheral tolerance is maintained by several mechanisms: (i) Immature APCs that have not been activated express only low levels of co-stimulators such as CD80 or CD86. When they interact with T lymphocytes by presenting antigen, clonal anergy is induced. Lymphocytes are then in a state of unresponsiveness, even when activated by mature APCs at a later time point. (ii) Repeated and persistent activation leads to activation-induced cell death, and thereby deletion of T cells. (iii) T cell

activation can be suppressed by regulatory T lymphocytes. (iv) Lymphocytes are tolerised by clonal ignorance, a state where lymphocytes do not encounter their cognate antigen due to physical barriers or developmental expression patterns.

1.2 Tumour Immunology

Tumours arise by uncontrolled proliferation of transformed body-own cells. The malignancy of tumours depends on the proliferative capacity of the transformed cells and the ability of these cells to invade host tissue and to metastasise to distant sites. In the early 20th century Paul Ehrlich was the first to postulate that cancer cells can be recognised and eliminated by immunologic responses (Ehrlich, 1909). Macfarlane Burnet proposed the concept of immunosurveillance in 1970 (Burnet, 1970). He postulated that the immune system would constantly scan the body for infected or transformed cells to then eradicate these. However, until today the role of immune surveillance in non-virus-associated tumour control is strongly debated (Dunn *et al.*, 2002; Qin and Blankenstein, 2004). It has clearly been shown that some virally induced tumours occur at a higher frequency in immunodeficient mice and patients. Examples in mice are the SV40 and polyoma viruses that only induce tumours in immunoincompetent animals (Tooze, 1973). Humans with immunodeficiencies are very susceptible to infections by Epstein-Barr virus (EBV), hepatitis B virus (HBV) and human papilloma virus (HPV), which often induce malignancies in patients (Purtilo *et al.*, 1981; Block *et al.*, 2003; Longworth and Laimins, 2004). These correlations and mouse experiments demonstrate the crucial role of T lymphocytes in rejecting virally induced tumours (Tevethia *et al.*, 1974; Cheever *et al.*, 1986). It is not clear however, how important T cell immunity is for the control of chemical, physical or spontaneous tumour development (Stutman, 1974; Johnsen *et al.*, 2001; Shankaran *et al.*, 2001).

In general, immune reactions against tumours involve the whole immune system. As part of the innate immune system, natural killer (NK) cells and macrophages exhibit anti-tumour reactivity. NK cells can kill tumour cells which have down-regulated MHC class I expression and therefore evade CTL responses. They can also kill cells to which tumour-specific antibodies (see below) have bound. Macrophages bind such antibodies as well. They bind via their Fc receptors (FcγRI) and subsequently kill the

opsonised tumour cells. Additionally, activated macrophages secret cytokines such as tumour necrosis factor (TNF) and interferon-γ (IFN-γ). Both cytokines have been shown to play an important role in anti-tumour immunity (Urban *et al.*, 1986; Watanabe *et al.*, 1989; Mumberg *et al.*, 1999). It is thought, however, that the adaptive immune system and especially the cell-mediated reactions play a more important role in anti-tumour reactions. CTLs play a crucial role in MHC class I dependent tumour cell killing. This has been shown in various mouse models such as immunisation-challenge experiments with virally and chemically induced tumours (Klein *et al.*, 1960; Boyse *et al.*, 1962). Furthermore, tumour infiltrating lymphocytes (TILs) can be isolated from some patients. These T cells show *in vitro* cytolysis of cancer cells (Schendel *et al.*, 1993; Kawakami *et al.*, 2000). The role of $CD4^+$ T lymphocytes in anti-tumour reactions is less clear. They play a role in activation of tumour-reactive CTLs, in establishing immunological memory, but they can also have a direct effect on tumour regression as cytokine producing cells (Frasca *et al.*, 1998; Zajac *et al.*, 1998; Toes *et al.*, 1999). Some $CD4^+$ cells, however, also show an inhibitory effect on anti-tumour reactions as regulatory T cells (Awwad and North, 1988). The role of B cells in anti-tumour immune reactions is also not well understood. They play a role in activation of T cells, especially $CD4^+$ cells, and as mediators of humoral responses. Anti-tumour antibodies have an indirect role in antibody dependent cell-mediated cytotoxicity against tumour cells, as described above, and by inducing complement-mediated cytotoxicity. However, the importance of such tumour-specific antibodies is debated, and in some models, B cells reduce CTL mediated anti-tumour responses (Qin *et al.*, 1998). Although many different anti-tumour reactions have been described in animal models and patients, the immune system is mostly not strong enough to overcome a growing cancer. Only very few case studies on spontaneous regressions can be found in the literature (Guthbjartsson and Gislason, 1995; reviewed in Snow and Schellhammer, 1982). One reason for this is the genetic instability of cancer cells that constantly produces new variants, of which some are able to evade the immune response. Examples are the down regulation of MHC molecules (Garrido *et al.*, 1997; Dudley *et al.*, 2005), the evolution of antigen-loss variants (Uyttenhove *et al.*, 1983; Yee *et al.*, 2002) and the occurrence of point mutations in targeted antigens (Lill *et al.*, 1992).

1.2.1 Tumour antigens

Antigens on tumours can be divided into two groups: (i) Tumour-specific antigens (TSA) are expressed on cancer cells only. (ii) Tumour-associated antigens (TAA) are found primarily in cancer cells, but are also expressed in normal tissue. Antigens of either group have been identified by several approaches. Many TSAs and TAAs have been identified by using tumour-reactive T cells, either from mice (Dubey *et al.*, 1997) or from humans, such as TILs isolated from tumour tissue of cancer patients (Boon and van der Bruggen, 1996). They are used to screen complementary DNA (cDNA) libraries (van der Bruggen *et al.*, 1991) or peptides eluted from MHC molecules of tumour cells (Cox *et al.*, 1994; Dubey *et al.*, 1997). Computerised algorithms have been developed to predict the peptide binding to MHC molecules derived from any protein (Boon and van der Bruggen, 1996). By this method, referred to as reverse immunology, potential antigens originating from mutations, known to have a transforming effect, can be identified. Another method to identify TAAs is the serological analysis of recombinant cDNA expression libraries of tumours (SEREX) (Old and Chen, 1998). In this method potential anti-tumour antibodies found in the serum of patients or animals are used to identify their target proteins.

1.2.1.1 Tumour-specific antigens

The generation of inbred mouse strains and their use in methylcholantrene induced carcinogenesis and subsequent tumour transplantation experiments gave the first evidence for the existence of TSA (Gross, 1943; Prehn and Main, 1957). In contrast to earlier experiments it was shown that the induced immunity was directed against tumour antigens of the transplanted cancer cells and not against foreign tissue itself (Woglom, 1929). The antigens involved in the rejection of tumours are also called rejection antigens. Due to the experimental model by which they have been described, they are also often referred to as transplantation antigens. Later experiments have shown most of these antigens not to be shared among different cancer cell lines. However, they were shown to be tumour-specific, meaning not to be present in any non-malignant cell. These non-shared, tumour-specific antigens arise through random mutations of different cellular genes, caused by physical or chemical carcinogens (Basombrio and Prehn, 1972; Monach *et al.*, 1995; Coulie *et al.*, 1995; Dubey *et al.*, 1997).

Many tumour antigens identified by tumour-reactive T cells are tumour-specific, mutant antigens. These antigens are recognised as foreign by the immune system. Therefore strong T cell responses against such mutant antigens can be mounted. Many TSA have been described for mouse models but also for individual human patients (Tab. 1). Transforming mutations often lie in mutational hot spots. Therefore, some of these antigens are shared among tumours of different patients. The fusion protein Bcr-Abl and mutant Ras are examples for such shared, mutated TSAs. Interestingly however, chemically induced fibrosarcomas did not show cross-protection in immunisation-challenge experiments with several tumour lines that shared the same Ras mutation (Carbone *et al.*, 1991). This reflects the situation in humans, where it is still not clear which role such shared TSAs play in tumour rejection, especially when they were identified by reverse immunology. With this approach, using *in vitro* peptide stimulation, T cells of a wanted specificity can often be found. However, their function *in vivo* is thereby not proven. Other mutations, as in p53, are not restricted to certain nucleotides. They therefore do not give rise to shared antigens. Any mutation inhibiting the function of p53 is likely to be transforming, as p53 is a tumour-suppressor.

Another group of tumour antigens which is recognised as foreign by the immune system are tumour antigens of viral origin. Several DNA viruses are associated with human cancers. The viral genomes encode proteins that lead to malignant transformation of host cells (Tab. 1). Several proteins of EBV are associated with B cell lymphomas and nasopharyngeal carcinoma (Rickinson *et al.*, 1992). The proteins E6 and E7 of HPV are associated with cervical cancer and others (Tindle, 1996). Also adenoviruses and polyoma viruses express transforming, so called early region genes (Gallimore and Turnell, 2001; Imperiale, 2001). All these antigens are tumour-specific as they are only expressed in tumours, not in normal tissue. Furthermore they are shared among patients with the same kind of tumour. Not only DNA viruses are connected to cancer, but also infections with RNA viruses show malignant transformations. Adult T cell leukaemia (ATL) can be induced by human T cell lymphotropic virus-1 (HTLV-1) (Matsuoka, 2003). As the transforming genes of RNA viruses, viral oncogenes, are very similar and sometimes even identical to cellular genes (Duesberg, 1983; Weinberg, 1996), the immune system is often tolerant of

these antigens. However, the immune system can elicit humoral and cellular responses towards the viral proteins (Coffin *et al.*, 1997).

1.2.1.2 Tumour-associated antigens

Shared tumour antigens are often thought to be of greater therapeutic value, as they are not only expressed in cancer cells of a certain individual, but in a wider range of patients. These shared antigens could therefore be used as a target by off-the-shelf immunotherapy (see 1.3). However, most of these antigens are not tumour-specific. They are tumour-associated, which means they are expressed in cancer cells, but also in non-cancer cells and often also in the thymus (Derbinski *et al.*, 2001; Gotter *et al.*, 2004). The latter point is important concerning negative selection of T cells (see 1.1.5) and also for positive selection of suppressor T cells (von Boehmer *et al.*, 2003). Consequently, immune responses against these antigens are rare and mostly not as strong as against mutated or viral tumour antigens.

TAA are non-mutated cellular proteins that are aberrantly- or over-expressed in tumour cells. They can be grouped according to expression patterns and molecular structure (Tab. 1) (Renkvist *et al.*, 2001; Schreiber, 2003). One group is formed by cancer-testis (oncospermatogonal) antigens. These proteins are normally only expressed in the MHC class I negative spermatocytes and spermatogonia of testis, but as recently shown, also in the thymus (Gotter *et al.*, 2004). These genes are found to be transcriptionally active in some cancer cells as well. The melanoma antigen encoding genes (MAGE) and New York-esophagus 1 (NY-ESO-1) are examples for cancer-testis antigens. A similar group of antigens is composed of proteins normally only expressed during foetal development. Also these carcinoembryonic (oncofoetal) antigens are aberrantly expressed in some tumours. The carcinoembryogenic antigen (CEA) and α-fetoprotein (AFP) are found in this group. Interestingly, newer studies have found both proteins to be expressed in non-malignant adult tissue as well (Kim *et al.*, 1992; Ohguchi *et al.*, 1998). Another group of TAA, the differentiation- or lineage-specific antigens, are expressed in tumour cells and in those cells and tissues, from which the tumour cells have originated. Most of the differentiation antigens are found in melanomas and haematopoietic cancers. Glycoprotein 100 (gp100), melanoma antigen recognised by T cells-1/melanoma

Table 1 | **Examples of human tumour antigens**

Tumour-specific antigens (TSA)		
Non-shared mutated antigens	CDK4 (cycline-dependent kinase 4)	(Wolfel *et al.*, 1995)
	p53	(Theobald *et al.*, 1995)
	HLA-A2-R170I	(Brandle *et al.*, 1996)
	β-catenin	(Robbins *et al.*, 1996)
	Caspase 8	(Mandruzzato *et al.*, 1997)
Shared mutated antigens	Ras	(Abrams *et al.*, 1996)
	Bcr-Abl (breakpoint cluster region-Abelson)	(Bocchia *et al.*, 1996)
Antigens of viral origin	E6 and E7 of HPV	(Kast *et al.*, 1994)
	EBNA 3 (EBV nuclear antigen 3)	(Burrows *et al.*, 1990)
	T antigen of human papovavirus JC	(Deckhut *et al.*, 1991)
	EA1 of adenovirus	(Urbanelli *et al.*, 1989)
Tumour-associated antigens (TAA)		
Cancer-testis antigens	MAGE-A1 (Melanoma antigen)	(van der Bruggen *et al.*, 1991)
	BAGE (B antigen)	(Boel *et al.*, 1995)
	GAGE (G antigen)	(Van den Eynde *et al.*, 1995)
	NY-ESO-1 (New York-esophagus 1)	(Jager *et al.*, 1998)
Carcinoembryonic antigens	CEA (carcinoembryogenic antigen)	(Tsang *et al.*, 1995)
	AFP (α-fetoprotein)	(Butterfield *et al.*, 1999)
Differentiation antigens	Tyrosinase	(Brichard *et al.*, 1993)
	MART-1/Melan-A (melanoma antigen recognised by T cells-1/melanoma antigen A)	(Kawakami *et al.*, 1994a)
	gp100 (glycoprotein 100)	(Kawakami *et al.*, 1994b)
	TRP-2 (tyrosinase related protein 2)	(Wang *et al.*, 1996)
	CD19 (cluster of diferentiation 19)	(Uckun *et al.*, 1988)
Clonal antigens	B cell receptor idiotype	(Miller *et al.*, 1982)
Over-expressed antigens	MUC-1 (mucin 1)	(Jerome *et al.*, 1991)
	HER-2/Neu (human epidermal receptor-2/neurological)	(Ioannides *et al.*, 1993)
	p53	(Theobald *et al.*, 1995)
	hTERT (human telomerase reverse transcriptase)	(Vonderheide *et al.*, 1999)
	G250 (glycoprotein 250)	(Vissers *et al.*, 1999)
	WT1 (Wilms' tumour gene)	(Oka *et al.*, 2000)
Glycolipids	diasialoganglioside GD3	(Cheresh *et al.*, 1984)

antigen A (MART-1/Melan-A), and tyrosinase are differentiation antigens found in melanocytes and melanoma cells. The B cell marker CD20 is also expressed on B cell lymphoma cells. A subgroup of differentiation antigens is formed by clonal antigens. These antigens are only expressed in a small group of cells from which the cancer arose. So far clonal antigens have only been described for B and T cell malignancies, as these cells have a clonal marker, their antigen receptor or idiotype. Finally, one group of TAAs is composed of widely expressed proteins that are over-expressed in tumours. This group includes the human epidermal growth factor receptor 2 (erbB-2, HER-2/Neu), which is expressed in some breast and ovarian cancers and other adenocarcinomas.

Most TAAs described so far, are presented as peptides in the context of MHC molecules. However, some TAAs do not need to be processed, but are directly exposed on the cell surface and can be recognised and bound by antibodies. Many of these latter TAAs are nevertheless processed by APC and presented in a MHC context. That is why for many TAAs to which antibodies exist also specific T cells, especially $CD4^+$ cells, have been described. HER-2/Neu and the renal cell carcinoma (RCC) antigen G250 are examples for this group of TAA (Kobayashi *et al.*, 2000; Vissers *et al.*, 2002). Finally, not only proteins and the associated peptides are recognised by the immune system, but also glycolipids and the glycosidic residues of glycoproteins can act as TAAs. Melanomas for example express high levels of gangliosides GM2, GD2 and GD3, to which patients often generate antibodies.

1.3 Cancer immunotherapy

In a review on cancer immunotherapy from 1929 W.H. Woglom wrote: “It would be as difficult to reject the right ear and leave the left ear intact, as it is to immunise against cancer” (Woglom, 1929).

In general, immunity can be achieved by active or passive immunisation, the activation and expansion of the endogenous immune repertoire or the transfer of exogenous immunoglobulins or immune cells, respectively. Passive immunisation is preferably used when patients are immunocompromised. Self-tolerance resembles such an immunocompromised state, and most TAAs are self-antigens. For this

reason, passive immunisation plays an important role in cancer immunotherapy, as discussed later in this chapter. Furthermore, immunisations can be categorised into therapeutic or prophylactic approaches. The common and effective immunisation against viral and bacterial pathogens is based on prophylactic vaccines. Due to the nature of the antigens, this approach only seems feasible for virally induced cancer (see 1.2.1.1). For other tumour entities, mostly active, therapeutic immunisations are being investigated.

In the beginning of the 20th century first trials were started to actively immunise patients against their own cancerous tissues (Bruns, 1887-1888; Coley, 1893; reviewed in Crispen, 1989). The patients were immunised with relatively crude tumour cell extracts and adjuvant, such as bacillus Calmette-Guérain, an attenuated strain of *Mycobacterium bovis*, or bacterial extracts such as Coley's toxin. Even today, where much more is known about the interactions of tumours and the immune system (see 1.2), very similar vaccination strategies are still followed (reviewed in Alexandroff *et al.*, 1999). Patients are immunised with autologous or allogeneic tumour cells that are either killed or inhibited in their proliferation. The capability to induce immunity can be augmented by using genetically modified tumour cells expressing stimulatory cytokines as granulocyte-macrophage colony stimulating factor (GM-CSF) or interleukin-12 (IL-12) and/or co-stimulatory molecules (Chen *et al.*, 1992; Schendel *et al.*, 2000; reviewed in Dranoff, 2004). Other strategies use purified TAAs to immunise. These include vaccination strategies with adjuvants, peptide pulsed DCs or antigen-transfected DCs (reviewed in Brossart *et al.*, 2001 and Nouri-Shirazi *et al.*, 2000). Furthermore, DNA encoding TAAs can be directly used as a vaccine (DNA vaccine) (Schreurs *et al.*, 1998) or in context of viral vectors (Zhai *et al.*, 1996). However, with the exception of an anti-idiotype DC vaccination trial with B cell lymphoma patients (Timmerman *et al.*, 2002), clinical results from vaccine trials have so far been disappointing (Rosenberg *et al.*, 2004). Many vaccination experiments in animals have shown an indirect correlation of tumour size at the first day of vaccination and therapeutic success.

Also for passive immunisation many different strategies have been analysed in the context of tumour immunotherapy. Some therapies using monoclonal antibodies (mAb) as transferred immune effectors have been approved for clinical use. One

example is Herceptin, a mAb directed against HER-2/Neu (see 1.2.1.2) (Drebin *et al.*, 1986; Bacus *et al.*, 1992). mAbs bound to tumour cells can activate NK cells and macrophages, can directly interfere with tumour growth by blocking stimulatory receptors, as for Herceptin, but can also be used to transport toxins to tumour cells (reviewed in Ghetie and Vitetta, 1994 and Reisfeld and Gillies, 1996). Similarly, bispecific antibodies can be used to direct CTLs to tumour cells (reviewed in Segal *et al.*, 2001). These mAbs contain two specificities and bind TAAs and CTLs at the same time. Passive immunisation does not only employ mAbs, but also living cells can be used to immunise. Adoptive cellular therapy is the transfer of cultured immune cells with anti-tumour reactivity into tumour-bearing hosts. The transfer of lymphokine activated killer (LAK) cells combined with systemic IL-2 treatment showed regression of metastases of different tumour entities in animal models (Lafreniere and Rosenberg, 1985; Shiloni *et al.*, 1986; Mule *et al.*, 1986). However, no major clinical impact could be seen in several trials (Rosenberg *et al.*, 1993; reviewed in Morse *et al.*, 2002). LAK cells are mainly NK cells generated by *in vitro* culture of peripheral blood lymphocytes (PBLs) with high IL-2 concentrations. A different approach is the transfer of tumour-specific T cells (see 1.3.1) (Hanson *et al.*, 2000).

All immunisation strategies described so far can be supplemented by a general stimulation of the host's immune system. In some cases such stimulation alone can lead to anti-tumour reactions. Stimulatory cytokines that induce proliferation and differentiation of T cells and NK cells can partially replace T helper cell functions during the priming phase of an immune response. IL-2 was shown to have a positive effect on survival and anti-tumour function of adoptively transferred T cells (Rosenberg *et al.*, 1994; Yee *et al.*, 2002). IFN-α is used for treatment of chronic myelogenous leukaemia (CML), where it shows immune stimulation but also direct effects on tumour cells (reviewed in Dranoff, 2004). The cytokines can either be applied systemically, intratumourally, or, as previously discussed, in form of cytokine secreting vaccine cells. Other immune system-stimulating approaches are intratumoural administrations of inflammatory substances such as bacillus Calmette-Guérain or anti-CD3 mAb. The mycobacterium activates macrophages and other effector cells and may function as an adjuvant to trigger a T cell response (Bohle, 2000).

1.3.1 Adoptive Immunotherapy

Adoptive immunotherapy is the transfer of immunological effector cells. These cells can be obtained from a donor or can be of autologous source. Furthermore, these cells can be of a single specificity, or target a rather broad range of antigens. The latter approach finds its application in allogeneic stem-cell transplantation (allo-SCT), an accepted curative strategy for the treatment of haematological tumours such as CML (Kolb *et al.*, 1990) and acute lymphoblastic leukaemia (ALL) (Slavin *et al.*, 1996). T lymphocytes play a crucial role in the observed graft-versus-leukaemia (GvL) effect (Maraninchi *et al.*, 1987; Kolb *et al.*, 1995; Falkenburg *et al.*, 1999). The nature of the rejection antigens involved is not fully understood. However, minor histocompatibility antigens play an important role, as GvL cannot be detected to such an extent when autologous bone marrow is transferred (Gale *et al.*, 1994; Horowitz *et al.*, 1990). The tumour eradication by allo-SCT, an introduction of a non-tolerised, tumour-reactive T cell pool into patients, indicates the possibilities that adoptive T cell therapies harbour.

Other successful applications of adoptive T cell therapy, in this case applying T cell pools with a defined specificity, are the treatment of cytomegalovirus (CMV)-mediated disease and EBV-positive B cell lymphomas in post-transplant immunosuppressed patients (Walter *et al.*, 1995; Rooney *et al.*, 1995). The infusion of *in vitro* expanded, virus-specific T cell lines protected the patients from these transplantation-associated diseases.

The application of adoptive T cell transfer for non-virus associated tumours has so far been of only partial success. The basic approach is to isolate TILs from patients, amplify the tumour-reactive T cells *in vitro* and finally transfer the effector cells back into the patient. In contrast to the treatment of virus-related diseases, this process is long, cumbersome and often unsuccessful for most tumour entities. This is partially due to the low numbers of TILs in the tumour tissue, their low affinity and avidity but also due to the anergic state of many tumour-specific T lymphocytes (Lee *et al.*, 1999). The only exception so far is melanoma, a tumour entity known for its immunogenicity. Here, TILs can be isolated from patients with a frequency of up to 50% (Lee *et al.*, 1999). Furthermore, one clinical trial using autologous TIL lines has shown objective clinical responses in half of the treated melanoma patients (Dudley

et al., 2002; Dudley *et al.*, 2005). The adoptively transferred TIL lines showed expansion of antigen specific clones *in vivo* and preferential trafficking to tumour sites. Additionally, re-isolated CTLs showed tumour cell lysis and cytokine production upon *ex vivo* co-culture with target cells (Dudley *et al.*, 2002). In order to apply adoptive immunotherapy to a broader range of tumours, a combination of gene transfer and adoptive T cell therapy seems promising. The approach is based on the transfer of anti-tumour specificity to autologous PBLs. This would allow an adoptive transfer of high numbers of autologous T cells, grafted with TCRs of any anti-tumour specificity.

1.4 Changing a T cell's specificity

1.4.1 Retroviral vectors

A vector for gene therapy has to be chosen according to the requirements of the application. T cells adoptively transferred to cancer patients must express the specific TCR in amounts that ensure the necessary avidity for anti-tumour effector functions. Furthermore, these tumour-reactive T cells should persist at least until all tumour cells have been eradicated, ideally as memory T cells circulating in the periphery, preventing any later outgrowth of residual cancer cells. The combination of gene therapy and adoptive cancer therapy therefore demands for a vector, which can efficiently transduce primary T lymphocytes. Within only a short time therapeutic numbers of tumour-reactive T cells for the adoptive transfer should be obtained. Furthermore, high transgene expression has to be guaranteed, to ensure high avidity of the gene-modified T cells. Finally, long lasting transgene expression is important for the persistence of memory T cells in patients. To date only retroviruses seem capable of fulfilling these demands (Mavilio *et al.*, 1994; Bunnell *et al.*, 1995; reviewed in Weber *et al.*, 2001).

The transduction efficacy of retroviruses is dependent on the transduction protocol and the viral envelope (env) proteins. The pseudotype of retroviral particles is determined by the *env* gene used to generate the producer cell line. These virus releasing cells contain all viral genes, encoding the viral enzymes and structural proteins. The *env* gene encodes the surface envelope glycoprotein gp70, responsible for the virus-host cell interactions prior to infection. The Gibbon ape leukaemia virus

(GaLV) and the mutated amphotropic 10A1 murine leukaemia virus (MLV) pseudotypes have been shown to be most effective in transducing primary human T lymphocytes (Bunnell *et al.*, 1995; Uckert *et al.*, 2000). As for the transduction method, many different protocols exist. These include pre-coating of the culture plates with virus (Darling *et al.*, 2000; Kuhlcke *et al.*, 2002), spinning the culture plates containing cells and virus, and multiple rounds of infection (Lamana *et al.*, 2001). For most methods, however, culture plates are coated with a recombinant fibronectin fragment (RetroNectin) that captures cells and virus particles and thereby allows high transduction rates due to high local concentrations of both cells and virus (Hanenberg *et al.*, 1996; Pollok *et al.*, 1998).

The transgene expression depends on *cis*-regulatory elements contained in the retroviral vectors. Also here, many studies describe the improvement and optimisation of retroviral gene transfer. Most retroviral vectors are based on the Moloney-murine leukaemia virus (MoMLV) backbone (Miller and Rosman, 1989). However, these vectors have been shown to be sensitive to transcriptional silencing, which is associated with methylation of the long terminal repeats (LTR) (Jahner *et al.*, 1982; Challita and Kohn, 1994; Wang *et al.*, 1998; Swindle *et al.*, 2004). These viral genome flanking regions contain the viral promoter and enhancer sequences. LTRs obtained from other murine retroviruses, such as the myeloproliferative sarcoma virus (MPSV), seem to be less prone to methylation and therefore allow long-lasting transgene expression (Hawley *et al.*, 1994; Pawliuk *et al.*, 1994; Kaneko *et al.*, 2001). The construction of chimeric viruses, which contain sequences of different viral origin, combines the advantages of several viruses.

Applying optimal promoters is the most obvious approach to enhance the expression rates of retroviral vectors. The promoters can be either part of the LTR, derived from different virus strains, or inserted as independent internal promoters. Strong promoters as the CMV promoter or the cellular phosphoglycerate kinase (PGK) promoter are often used as internal promoters (Cooper *et al.*, 2000). However, transcription rates are usually not as high as for viral LTR promoter/enhancer pairs and there are reports on low transgene expression due to promoter interference (see 4.1.1).

In order to enhance transgene expression also other DNA-elements have been introduced into viral backbones. These include the Woodchuck hepatitis virus post-transcriptional regulatory element (PRE) (Schambach *et al.*, 2000), which is a *cis*-acting sequence that enhances gene expression, most probably by promoting polyadenylation, nuclear export and translation initiation (Donello *et al.*, 1998; Huang *et al.*, 1999). Inserted splice donor and acceptor sites act similarly, as the binding of the splicing machinery also leads to mRNA stability and transport into the cytosol (Matsumoto *et al.*, 1998; Luo and Reed, 1999; Hildinger *et al.*, 1999). Furthermore, the spliceosome enhances the initiation of translation. The constitutive RNA transport element (CTE) also promotes nuclear export, however, of unspliced mRNA. It has been isolated from the Mason-Pfizer monkey virus. Also in the context of retroviral vectors it has been demonstrated to exert mRNA transport capabilities (Bray *et al.*, 1994; Schambach *et al.*, 2000).

Low transgene expression often occurs when vectors integrate into transcriptionally silent chromosomal regions. The introduction of insulator sequences reduces these chromosomal position effects (Agarwal *et al.*, 1998; Dang *et al.*, 2000). Insulator elements often originate from locus control regions (LCR). As their name indicates, these sequences control the transcriptional activity of an entire gene locus and confer tissue-specific, position independent, and copy number dependent expression of genes. Their prototype is the β-globin LCR (Grosveld *et al.*, 1987), which has been successfully employed in the context of retroviral gene expression (Novak *et al.*, 1990; May *et al.*, 2000). Other LCRs have been isolated from the human CD2 gene (Greaves *et al.*, 1989) and the TCR α/δ locus (Diaz *et al.*, 1994). As for the β-globin LCR, also the function of the CD2 LCR has been investigated in the context of retroviral vectors (Kowolik *et al.*, 2001; Indraccolo *et al.*, 2001), and its activity was dissected into an enhancer and a LCR region.

1.4.2 Transfer of T cell receptors

The specificity of a T cell is solely determined by its TCR α and β-chains. A transfer of these two chains is sufficient to transfer specificity. This was shown by Dembic and colleagues in 1986 (Dembic *et al.*, 1986). They achieved the transfer of specificity by cell fusion. Today T lymphocytes can be redirected by TCR gene transfer. Based on

studies to optimise retroviral vectors for transduction of primary human T lymphocytes, Clay and colleagues were the first to describe such TCR transfer into primary T cells (Clay *et al.*, 1999). To date several groups have redirected murine and human T cells towards specificities against viral, cancer, and model antigens (see Appendix; reviewed in Schumacher, 2002). In these studies many different retroviral vectors have been employed. Early publications describe gene transfer and the analysis of the redirected cells *in vitro* with regards to specificity and effector functions (Clay *et al.*, 1999; Cooper *et al.*, 2000; Fujio *et al.*, 2000). Further studies focus on the *in vivo* behaviour of the modified T cells. Following the transfer of murine T cells grafted with an Influenza virus-specific TCR, Kessels and colleagues could show proliferation upon viral infection, homing to effector sites, virus clearing and also rejection of tumours expressing the viral antigen (Kessels *et al.*, 2001). In another study Chamoto and colleagues demonstrated tumour eradication in mice treated with adoptively transferred redirected ova-specific T cells and cyclophosphamide (Chamoto *et al.*, 2004).

1.4.3 Transfer of chimeric antigen receptors

Similarly to TCRs, also T-bodies/chimeric antibody receptors (CAR) can transfer new specificities to T lymphocytes (reviewed in Eshhar, 1997 and Hombach *et al.*, 2002). CARs are hybrid receptors composed of the antigen binding fragment of an antibody and a signalling domain. These two functions, normally located on two different molecules of the TCR/CD3 complex, are combined into a single chimeric molecule. The antigen recognition domain is mainly composed of a single-chain variable fragment (scFv) obtained from monoclonal mouse antibodies. The scFv can be fused to many different combinations of transmembrane, hinge and signalling domains originating from different molecules (reviewed in Sadelain *et al.*, 2003). The ITAM containing intracellular domains of the CD3 ζ-chain or the Fcγ receptor (FcRγ) of the FcεRI are mainly used for signalling functions.

The first description of a CAR was published by Eshhar and colleagues (Eshhar *et al.*, 1993). They described the transfer of hapten-specificity to a mouse CTL hybridoma. Later, several studies have shown *in vivo* efficacy of murine CTLs redirected by tumour-specific CARs (Kershaw *et al.*, 2002; Cooper *et al.*, 2005).

Brentjens and collegues could for the first time show *in vivo* anti-tumour reactivity of redirected human T lymphocytes after transfer into tumour-bearing mice (Brentjens *et al.*, 2003). They grafted PBLs with a CD19-specific CAR and could demonstrate tumour rejection.

1.5 Renal cell carcinoma

Renal cell carcinoma (RCC) is the most prevalent form of malignancies of the kidney, with 85% of all kidney neoplasms. It accounts for 3% of all adult cancers (Schendel *et al.*, 1997). The primary treatment is radical nephrectomy. However, only 60 - 70% of the patients can be cured, even if diagnosed at an early state. This is due to frequent early dissemination of cells from the primary tumour, even though metastases often only appear several years later. The 5-year survival rate of patients with advanced metastatic disease lies at less than 15% and many patients die within one year. To date no effective treatment for metastatic RCC is known, as the tumour cells are relatively radio-, hormone- and chemoresistant. For this reason, alternative treatment strategies are under investigation.

RCC is considered to be one of the few immunogenic tumours besides melanoma. RCC cells are thought to be able to activate cells of the immune system to mount an anti-tumour response. One indication is the rare occurrence of spontaneous remission of metastatic lesions (Guthbjartsson and Gislason, 1995). However, the frequency is low at 0.5 to 1%. A stronger indication is the partial success of several clinical immunotherapy trials. Many different approaches have been undertaken. These include general immune system activating strategies such as systemic cytokine treatment (IL-2, IFN-α, and combinations) (reviewed in Bleumer *et al.*, 2003) and allo-SCT (Childs *et al.*, 2000; Rini *et al.*, 2002). The transplantation trials have shown partial and also complete responses in some patients. Also RCC antigen-specific approaches such as vaccinations with genetically-modified allogeneic tumour cells (Pohla *et al.*, 2000; Schendel *et al.*, 2000), with non-modified or genetically-modified autologous tumour cells (Veelken *et al.*, 1997; Simons *et al.*, 1997) or tumour lysate pulsed DCs (Holtl *et al.*, 2002; Oosterwijk-Wakka *et al.*, 2002) have been investigated. The phase I clinical trials however, only rarely showed objective clinical responses (Simons *et al.*, 1997; Holtl *et al.*, 2002).

The nature of the anti-RCC immune response is not yet clear. Nevertheless, abundant cellular infiltrates are observed in many tumours (Heinemann *et al.*, 1987; Storkel *et al.*, 1992), indicating that the cellular immune system might be involved in the tumour regressions observed. The infiltrates are composed primarily of T lymphocytes of both CD4 and CD8 lineage, but also macrophages and NK cells have been identified. In a few cases *in vitro* cultivation of TILs allowed the establishment of tumour-reactive T cell lines and clones (Belldegrun *et al.*, 1988; Schendel *et al.*, 1993; reviewed in Schendel *et al.*, 1997). Taken together, these observations and studies deliver a rationale for immunotherapy against RCC.

However, compared to the other immunogenic tumour entity, melanoma, only few RCC antigens have so far been identified (Tab. 2). Of the antigens known, only one is widely expressed. G250/MN/carbonic anhydrase 9 (CA IX) is expressed in 75 to 85% of all RCC types and only weakly expressed on the epithelium of larger bile ducts and on gastric mucosal cells (Oosterwijk *et al.*, 1986; Grabmaier *et al.*, 2000). The G250 antigen is a 49.6 kDa transmembrane protein, identical to the cervix carcinoma associated antigen MN. The function of the protein has not been clarified so far. The murine monoclonal antibody recognising G250 has been adopted in several clinical trials (Steffens *et al.*, 1999; Bleumer *et al.*, 2004). Other investigations have identified antigenic peptides of G250 which are recognised by $CD8^+$ and $CD4^+$ T lymphocytes (Vissers *et al.*, 1999; Vissers *et al.*, 2002). Appart from isolated TIL clones for which the cognate antigen has been identified, many “orphan” TILs have been established and analysed. The TIL-26 clone isolated by Schendel and colleagues recognises three RCC cell lines: the autologous line RCC-26 and the heterologous lines RCC-10 and 22 (Schendel *et al.*, 1993; Schendel *et al.*, 1997). However, further RCC lines and other tumour cell lines were not recognised. It is therefore not clear whether TIL-26 recognises a shared tumour antigen. A recently isolated TIL (TIL-53) has shown more promising results in preliminary *in vitro* studies. The majority of RCC lines tested, but also other tumour cell lines, were able to stimulate TIL-53 cells (E. Nößner, personal communication). Both TCRs are therefore good candidates for T cell redirection experiments.

Table 2 | **Renal cell carcinoma antigens**

Tumour antigen	Expression pattern	Ref.
Adipophilin (adipose differentiation-related protein)	RCC samples carcinomas, melanoma, CML; adipocytes and macrophages	(Schmidt *et al.*, 2004)
EHD2 (Eps15 homology domain-containing protein 2)	4 of 5 RCC samples analysed broadly expressed in malignant and non-malignant tissues	(Dorrschuck *et al.*, 2004)
EphA3 (Ephrin receptor)	31% of RCC samples analysed melanoma, carcinimas, and sarcomas; strong expression in retina, and foetal brain, significant expression in bladder, prostate, and cancer	(Chiari *et al.*, 2000)
FGF-5 (fibroblast growth factor-5, post-translational protein spliced peptide)	6 of 10 RCC lines analysed several adenocarcinoma lines, and low expression in brain and kidney	(Hanada *et al.*, 2001)
G250/MN/CA IX (carbonic anhydrase IX)	75 -85% of RCC samples weakly on epithelium of larger bile ducts and on gastric mucosal cells	(Grabmaier *et al.*, 2000)
mutant HLA-A2	patient- and tumour-specific	(Brandle *et al.*, 1996)
mutant hsp70-2 (heat shock protein-70)	patient- and tumour-specific	(Gaudin *et al.*, 1999)
iCE (alternative open reading frame of intestinal carboxyl esterase)	3 of 4 RCC samples analysed broadly expressed in non-malignant tissues	(Ronsin *et al.*, 1999)
KIAA1440	patient- and tumour-specific	(Zhou *et al.*, 2005)
alt.M-CSF (alternative open reading frame of macrophage colony-stimulating factor)	6 of 10 RCC samples analysed strong expression in the proximal tubule epithelium, hepatocytes and few cells of the duodenal submucosa	(Probst-Kepper *et al.*, 2001)
PRAME (Preferentially expressed antigen of melanoma)	40% of RCC samples analysed (mRNA) broadly expressed in malignant and non-malignant tissues	(Neumann *et al.*, 1998)
RAGE1 (Renal tumour antigen 1)	1 of 57 RCC samples analysed several malignant tissues and retina	(Gaugler *et al.*, 1996)
RU2 (antisense transcript)	10 of 10 RCC samples analysed several malignant and non-malignant tissues	(Van Den Eynde *et al.*, 1999)
SART-2 (Squamous cell carcinoma antigen recognized by T cells-2)	5 of 9 RCC samples mRNA expression is several non-malignant tissues	(Nakao *et al.*, 2000)

1.6 Outline of this thesis

The aim of this thesis was to lay the basis for an adoptive T cell transfer therapy for RCC. For this, two aspects had to be dealt with: 1) optimising retroviral gene transfer into human PBLs and 2) transferring a TCR and analysing the redirected PBLs in detail.

In order to successfully transfer TCR genes to PBLs, the retroviral transfer system has to be efficient with regard to transduction and transgene expression. Previous work in our lab has helped to improve transduction efficiencies, studying retroviral pseudotypes and virus production conditions. In this thesis emphasis was laid on the improvement of transgene expression (Chapter 2). The objective was to construct a retroviral vector that guarantees high und durable transgene expression, suitable for TCR transfer. For this purpose different *cis*-regulatory elements were compared:

- On the one hand retroviruses were constructed using the Moloney murine leukaemia virus (MoMLV) long terminal repeat (LTR) or the myeloproliferative sarcoma virus (MPSV) LTR.
- Additionally, the impact of the woodchuck hepatitis virus post transcriptional regulatory element (PRE) and the T cell specific CD2 enhancer were investigated in the context of the MPSV and MoMLV LTRs, respectively.

The performance of the vectors was analysed in both mouse and human cells. Furthermore, T cell lines and primary T lymphocytes as well as fibrosarcoma cell lines were used for expression analysis. The expression of the green fluorescent protein (GFP) was used as a read-out system for transduction efficiency and transgene expression.

For the second part of this thesis only two different retroviruses were used: the vector that performed best in GFP expression analysis and a standard MoMLV-based vector for comparison. The GFP gene in the retroviral vectors was exchanged with TCR α and β-chain genes (Chapter 3). The TCR genes were isolated from TIL-26, a previously described T cell clone obtained from a RCC TIL culture. T cell lines and PBLs of several donors were transduced with TCR-26 retroviruses.

- Firstly, TCR-26 surface expression was investigated.
- Functional tests were performed with the redirected PBLs. They included cytokine secretion and cytotoxicity assays upon co-culture with tumour cell lines.
- Finally, the performance of the redirected PBLs was compared to the originally isolated TIL-26 clone.