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Strategies for Antigen-Specific Immunomodulation in Experimental Organ Transplantation

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by

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SELECTED OWN PUBLICATIONS

PUBLICATION 1:
Otto C, Gasser M, Waaga-Gasser AM, Rohde AC, Lenhard M, Jost S, Gassel HJ, Ulrichs K, Timmermann W. Prolongation of small bowel allograft survival with a sequential therapy consisting of a synthetic MHC class II peptide and temporarily low-dose cyclosporine A. *Human Immunol* 2002; 63: 880-887

PUBLICATION 2A:
Sitaru AG, Timmermann W, Ulrichs K, **Otto C**. Hierachical immunogenicity of donor MHC class I peptides in allotransplantation. *Human Immunol* 2002; 63: 871-879

PUBLICATION: 2B:
Sitaru AG, Timmermann W, Ulrichs K, **Otto C**. Allogeneic core amino acids of immuno-dominant allopeptide are important for MHC binding and TCR recognition. *Human Immunol* 2004; 65: 817-825

PUBLICATION 3:
Gassel HJ, **Otto C**, Gassel AM, Meyer D, Steger U, Timmermann W, Ulrichs K, Thiede A. Tolerance of rat liver allografts induced by short-term selective immunosuppression combining monoclonal antibodies directed against CD25 and CD54 with subtherapeutic cyclosporine. *Transplantation* 2000; 69: 1058-1067

PUBLICATION 4:
Gassel HJ, **Otto C***, Klein I, Steger U, Meyer D, Gassel AM, Ulrichs K, Thiede A. Persistence of stable intragraft cell chimerism in rat liver allografts after drug-induced tolerance. *Transplantation* 2001; 71: 1848-1852

PUBLICATION 5:
Meyer D, Löffeler S, **Otto C**, Czub S, Gassel HJ, Timmermann W, Thiede A, Ulrichs K. Donor-derived alloantigen-presenting cells persist in the liver allograft during tolerance induction. *Transplant Int* 2000; 13: 12-20

PUBLICATION 6:
Otto C, Kauczoc J, Martens N, Steger U, Möller I, Meyer D, Timmermann W, Ulrichs K, Gassel HJ. Mechanisms of tolerance induction after rat liver transplantation: Intrahepatic $CD4^+$ T cells produce different cytokines during rejection and tolerance in response to stimulation. *J Gastrointest Surg* 2002; 6: 455-463

* contribute equally

DEFINITIONS

A major cause of transplant rejection is the activation of T lymphocytes. The antigens mainly responsible for this activation are found on cells of the graft and variously termed allogeneic MHC molecules, allo-MHC-molecules, or alloantigens. The immune response triggered by an allogeneic organ, i.e. an organ from an unrelated donor, is an alloimmune response. It can be divided into three phases (Fig. 1).

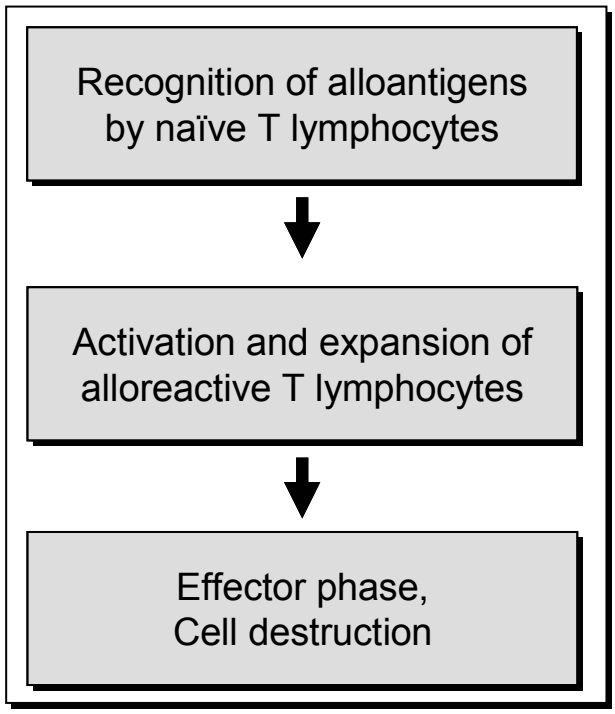

FIGURE 1: THE THREE PHASES OF THE ALLO RESPONSE. In phase 3, the effector phase, rejection leads to destruction of graft cells by cells of both the *acquired* and *innate* immune systems (regarding phases 1 and 2 see also Fig. 2).

Host T lymphocytes recognized by alloantigens are termed alloreactive T lymphocytes. There are two distinct pathways of alloantigen recognition: (1) The direct pathway, where alloreactive T lymphocytes recognize the allo-MHC-molecules on the surfaces of the passenger leukocytes transferred with the graft and are directly activated by them if they receive the necessary costimulatory signals. (2) The indirect pathway, where allogeneic MHC-molecules are processed by antigen presenting cells (APC) of the host and presented as allo-MHC-peptides in self-MHC-class-II molecules. This pathway was first described in 1982 by Lechler et al. (J Exp Med 1982; 155: 31).

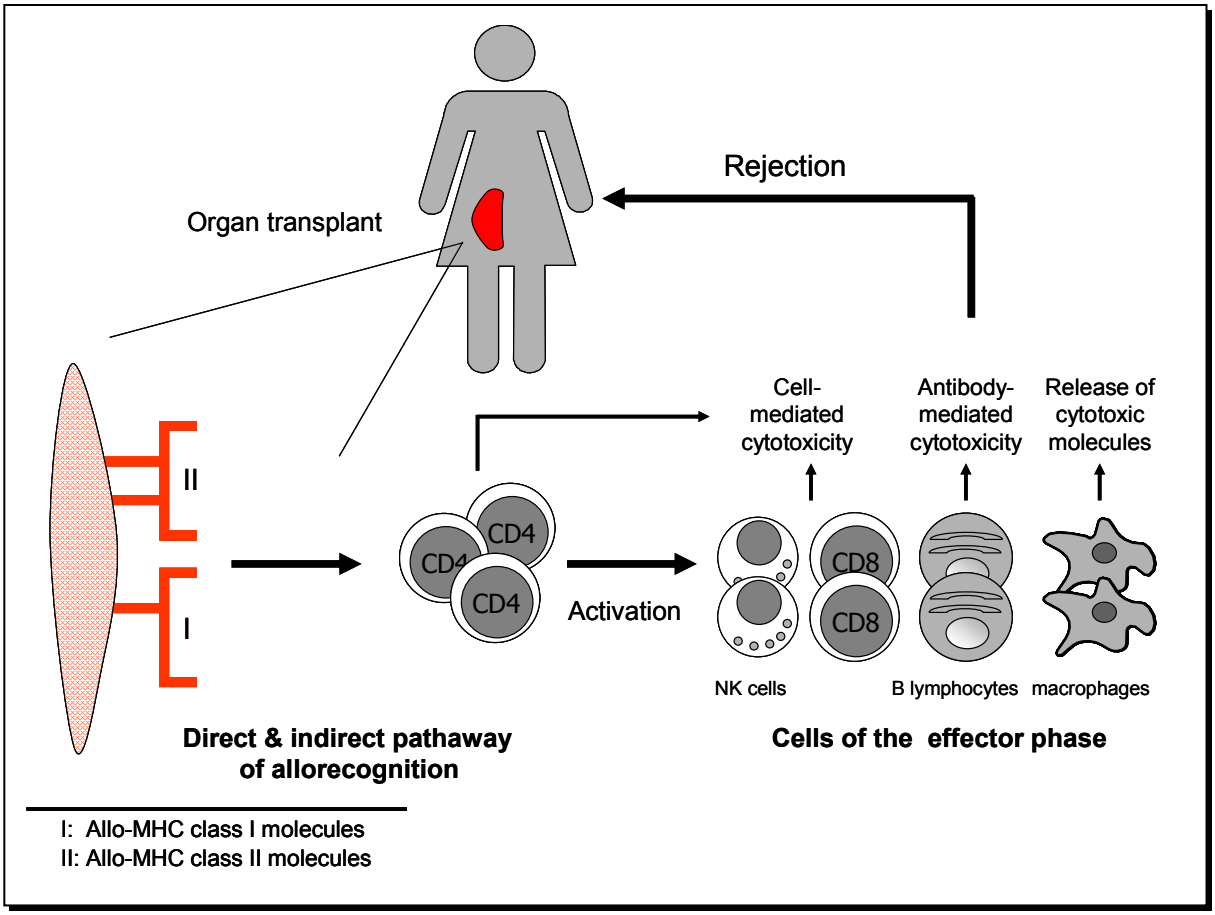

FIGURE 2: OVERVIEW OF THE CELLULAR MECHANISMS LEADING TO REJECTION. The figure highlights the three major components of the alloimmune response: (1) alloantigens, the main triggers of the alloimmune response; they can be either MHC-class-I or class-II molecules. (2) Alloreactive $CD4^+$ helper T lymphocytes (termed $CD4^+$ T lymphocytes in the following), which are activated following alloantigen recognition. (3) Cells of the effector phase, which are activated by the alloreactive $CD4^+$ T lymphocytes. These include cells of the adaptive immune system, B-lymphocytes, and cytotoxic $CD8^+$ T lymphocytes (termed $CD8^+$ T lymphocytes in the following), as well as cells of the non-adaptive immune system, such as macrophages and natural killer (NK)-cells. The effector phase is characterized by progressive cell destruction leading to the loss of graft function [1]. The mechanisms of direct and indirect alloantigen recognition are shown in Figs. 2.2 and 2.3.

1. SUMMARY

Transplant rejection is an immune response predominantly mediated by T lymphocytes. It is based on the immune system's ability to distinguish between "self" and "nonself". The main stimuli are the highly polymorphic allo-MHC-molecules coded by the major histocompatibility complex (MHC) and the allo-MHC-peptides processed from such allo-MHC-molecules by antigen presenting cells.

The immunosuppression concept is based on the targeted suppression of the host immune system. At present so-called immunosuppressive agents are used to inhibit graft rejection. This is not however without risk for patients, since long term suppression of the immune system increases the susceptibility to infections and malignant diseases. These drawbacks underscore the need for immunomodulating approaches that allow antigen-specific suppression of the alloimmune response without impairing the immune protection necessary for life. Two specific therapeutic concepts described here as ideal therapy based (1) on the modulation of the immune response with allo-MHC peptide variants and (2) regulatory $CD4^+$ T lymphocytes.

The effects such allo-MHC peptides have on the host immune system can be analyzed in detail because they can be produced synthetically. Studies have shown that allo-MHC-peptides possess both immunostimulatory and inhibiting properties. Our own data indicate they have a synergistic effect on immunosuppressive agents. In animal models preoperative sensitizing of the prospective host with the immunostimulatory peptide RT1.B2 in combination with short-term immuno-suppression was shown to prolong graft function to between 65 and 100 days. This phenomenon is known as "linked unresponsiveness" and cannot be achieved when each substance is given alone.

It is not at present possible to predict under which conditions the inhibiting properties of such peptides take effect. The amino acid sequences of allo-MHC-peptides are therefore purposely changed so as to modulate the properties of alloreactive T lymphocytes.

Unlike autoimmune diseases, the alloimmune response does not appear to be based on individual peptide antigens. To investigate which allo-MHC-peptide antigens dominate in the alloimmune response, we examined seven different peptides that are identical to certain areas of the MHC-class-I-molecule of haplotype $RT1^u$ (the haplotype of the Wistar-Furth [WF] rat), in Lewis (LEW)-rats with the haplotype $RT1^l$ were tested for their ability to trigger an immune response. These MHC-class-I-peptides represent the area in the $RT1.A^u$ molecule that differs from the $RT1.A^l$ molecule, i.e. the amino acids found here are different and must be responsible for the alloreactivity.

Of the seven allo-MHC-class-I-peptides investigated, the P1 peptide induced an immunodominant T-cell response leading to graft rejection. This important finding appears to reduce to a few the number of candidate allo-MHC-peptide antigens. In order to produce variants of the immunodominant P1 peptide, the three amino acids in the MHC-class-I-molecules of WF were sequentially replaced by the three amino acids occupying the same position in the MHC-class-I-molecule of LEW rat. With A1.5 a promising peptide variant was identified that delays graft rejection in an antigen-specific manner. The effective mechanism of A1.5 has been studied in detail: It displaces the original P1 peptide from the binding groove of MHC-class-II molecules.

A further attractive application of allo-MHC-peptides and/or their variants is the generation of regulatory cells. Regulatory T lymphocytes are promising mediators of peripheral tolerance. They or their secreted interleukins should make it possible to demonstrate changes in the immunological situation following transplantation. Reliable molecular-immunological markers of graft tolerance would be of great importance in clinical practice, since current assays do not always deliver unequivocal results.

The effect of a limited alloimmune response on the generation of regulatory cells was studied in immunologically active liver grafts. The liver actively modulates the immune system and after transplantation induces its own acceptance. If subjected to antigen stimulus, $CD4^+$ $CD45RC^{neg}$ hepatic T lymphocytes isolated from tolerated liver grafts produce the immunoregulatory Th2 interleukin (IL)-13. Another hepatic $CD4^+$ T-lymphocyte subpopulation of $CD45RC^{pos}$ phenotype lacks this property, as do $CD4^+$ $CD45RC^{neg}$ T lymphocytes of the spleen. Although no data are yet available on the immunomodulating effect of IL-13 in liver transplants, demonstration of this cytokine in combination with the relevant hepatic T-cell population can serve as a marker of stable tolerance.

At present clinical organ transplantation is the sole therapy for irreversibly damaged organs. While its success is due in large part to clinical application of the immunosuppression concept, its potentially grave side effects underscore the need for new strategies. The experimental approaches presented here for antigen-specific immunomodulation with allo-MHC-peptides and regulatory T lymphocytes could contribute to the development of both new diagnostic concepts and an ideal therapy.

2. INTRODUCTION

Organ transplantation has developed worldwide into a successful procedure for treating patients with irreversibly damaged organs [2]. Although its success can be attributed in large part to the optimization of transplantaiton surgery, organ preservation and tissue typing, the major part of its success is undoubtedly due to effective application of the immunosuppression concept [3, 4].

2.1 THE IMMUNOSUPPRESSION CONCEPT AND THE IDEAL THERAPY

The immunosuppression concept is based on the targeted suppression of the host's immune system. Application of the appropriate immunosppressive agents inhibits the immune response triggered by the graft and thus preserves the viability of the graft [5]. In the past 40 years the immunosuppression concept has given rise to effective therapies that are especially successful at ensuring short-term graft function. In the majority of tranplant centers, the current one-year survival rates for kidney, liver, lung and pancreas grafts is between 80% and 90% [6].The long-term maintenance of organ function, by contrast, remains a major problem that the current immunosuppression concept is largely unable to address. The chronic rejection that occurs months to years after transplantation[1, 2] does not respond to treatment with currently available immunosuppressive agents [7, 8, 9]. Moreover, suppress not only the graft-induced immune response, but the immune system's ability to fight infection and maignant disorders as well [10, 11]. They also have serious side effects such as nephro- and neurotoxicity. Some of them, especially the calcineurin inhibitors, are thought to promote chronic rejection [12].

[1] Gassel HJ, **Otto C**, Gassel AM. Chronic rejection in the rat liver transplantation model. *Graft* 2002; 5: 149-152

[2] Meyer D, **Otto C**, Gasser M, Heemann U, Ulrichs K, Thiede A. Chronic rejection after rat liver-small bowel transplantation. *Graft* 2002; 5: 135-140

While clinical trials are currently examining novel strategies for lessening the side effects of immunosuppresive agents [13, 14], the search continues for an ideal therapy based on the immunosuppression concept[3] (Fig. 2.1). Even though progressive rejection is a multicellular event [15], the alloreactive $CD4^+$ T lymphocytes play a crucial role in the initiation of rejection [16]. An ideal therapy should therefore inhibit the graft induced immune response in an antigen-specifc manner, be free of side effects, prevent the alloantigen-specific processes leading to chronic rejection [17] and promote the cellular and molecular mechanisms leading to tolerance. This would attain the holy grail of transplantation research, the induction of graft-specific tolerance [18, 19]. Although the specfications of an ideal therapy are very concrete, strategies for its attainment are the subject of intense debate.

It is the goal of the present study therefore to further investigate two concepts for antigen-specific immunomodulation, one employing allo-MHC-peptide variants, the other regulatory $CD4^+$ T lymphocytes (Chapter 2.4).

[3] **Otto C**, Timmermann W, Sitaru G, Jost S, Gassel HJ, Ulrichs K. Modulation of T cell reactivity with MHC peptides: A strategy for selective inhibition of the T cell response to allografts? *Transplantationsmedizin* 2001; 13: 21-31

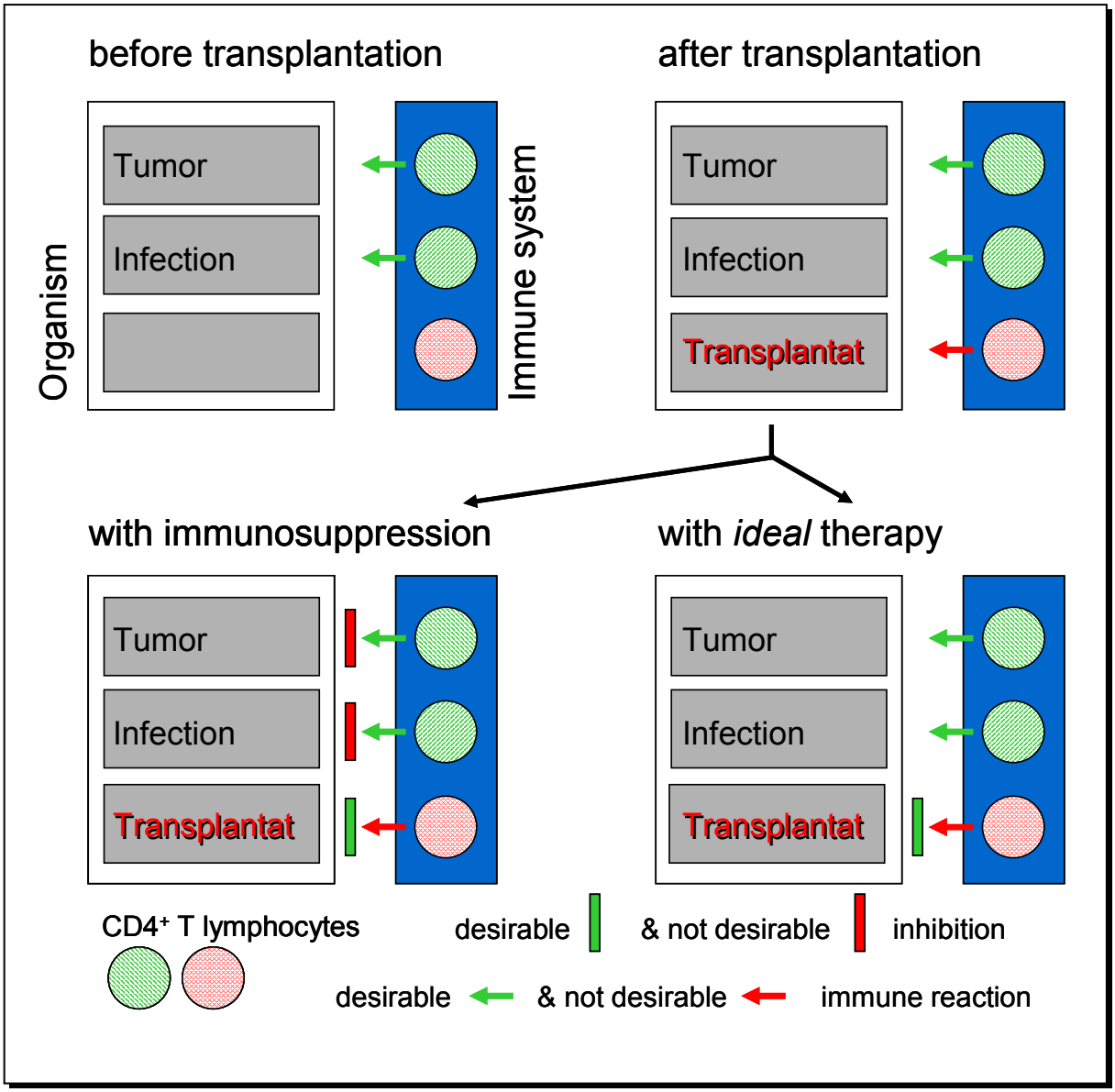

FIGURE 2.1: THE IMMUNOLOGICAL SITUATION BEFORE AND AFTER TRANSPLANTATION. At present the graft-induced immune response is suppressed by general immunosuppression. The goal of the ideal therapy is to selectively suppress the immune response to the foreign graft without impairing the immune system's vital ability to protect against infection and tumors. The ideal therapy is based on the antigen-specific suppression of the activation of the $CD4^+$ T lymphocytes participating in graft rejection.

2.2 HISTORY OF THE IMMUNOSUPPRESSION CONCEPT

Although the conceptional and experimental foundations of organ transplantation have their origins in the second half of the 19th century [20], it was not until

December 1954 that Murray et al. performed the first successful kidney transplant between identical twins [21]. Only when the same team could show that the immune barrier can be therapeutically overcome did transplantation become an attractive form of therapy [22, 23].

A special milestone in the development of the immunosuppression concept was the clinical introduction of cyclosporine A **(CsA)** (Sandimmun®) in 1980, which represented an unprecedated revolution in organ transplantaiton: Within 10 years the number of transplantations performed annually had increased by tenfold and the kidney transplantaiton survival rate in the first year after transplantaiton had risen to almost 80% [24]. CsA, like the bacterial agent tacrolimus (Prograf®) introduced in 1984, suppresses the activation of NFAT-dependent genes, such as interleukin (IL)-2, and thus is already effective in the early phase of T-cell activation [25, 26]. Despite their immunosuppressive potency, the use of calcineurin inhibitors is today now challenged due to the severity of their side effects [27].

At present the most successful monoclonal antibody (mAb) in transplantation medicine is the anti-IL-2 receptor antibody basiliximab (Simulect®). This antibody binds with high afinity to the α-chain of IL-2-receptors and thus suppresses IL-2 binding [28]. The current clinical successs of Simulect are highly promising, but only the future will reveal its true strengths and weaknesses [29]. An important finding of experiments with mAb is that while they represent a rational and effective supplement to immunosuppressive therapy, their use is not unaccompanied by side effects [30, 31].

All current efforts are concentrated on optimization of the immunosuppression concept to achieve an optimum of immunosuppressive efficacy with a minimum of side effects. A lively discussion is ongoing within the transplantation community regarding the possibility of individualized immunosuppression aimed at reducing the immunosuppressive dose to the necessary minumum [32]. It is agreed within the community that new therapeutic approaches are needed for prevention of chronic rejection [33, 34].

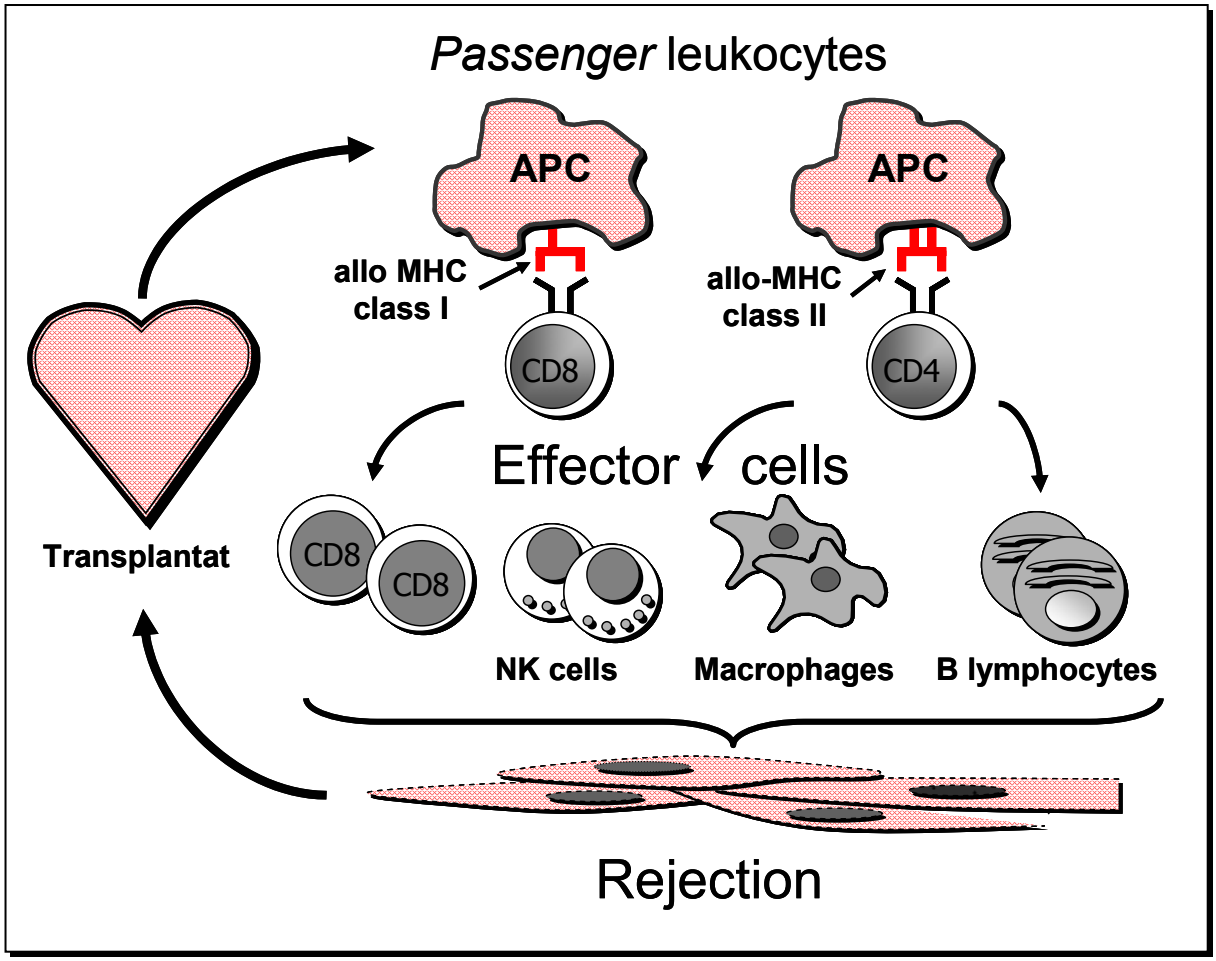

FIGURE 2.2: THE DIRECT PATHWAY OF ALLOANTIGEN RECOGNITION. The so-called *passenger leukocytes* transferred with the organ transplant activate alloreactive $CD4^+$ and $CD8^+$ T lymphocytes [35]. The activated $CD4^+$ T lymphocytes induce various effector cells participating in the rejection, among them NK cells, macrophages and B lymphocytes. This form of T-cell activation, in which up to 10% of the T lymphocytes are involved, is responsible for acute rejection. The basis of the indirect pathway of alloantigen recognition is the ability of the T-cell receptor to recognize allo-MHC-molecules as antigens on the cell surface. The colors indicate cells of different MHC-haplotypes: cells of the allogeneic graft are shown in red, host cells in grey.

2.3 THE IMMUNOBIOLOGY OF REJECTION

The antigens responsible for graft rejection are found both on the foreign graft and on its so-called passenger leukocytes (Fig. 2.2). Rejection is caused by the genetic incompatibility between the donor and host and was first described by Sir Peter Medawar in 1944 [36]. The surface proteins, the MHC-molecules, coded by the genes of the MHC are the most potent stimulus of the host immune system [37, 38].

The greater the incompatibility between the donor and host MHC proteins, the more rapid and intense the graft rejection [39]. Complete MHC-compatibility however does not necessarily mean rejection will not occur, since incompatibilities among the so-called minor MHC antigens [40] can lead to delayed rejection [41].

Upon transplantation, MHC-molecules, whose task is the presentation of foreign and self structures in the form of peptides, themselves become antigens. Allo-MHC-molecules are recognized by alloreactive T lymphocytes as intact molecules on the surfaces of passenger leukocytes. This direct pathway of alloantigen recognition leads to activation of both $CD4^+$ and $CD8^+$ T lymphocytes (Fig. 2.2). When, conversely, allo-MHC-molecules of host APC are processed to allo-MHC-peptides and presented in self-MHC-class-II-molecules on the cell surface, alloreactive $CD4^+$ T lymphocytes are activated. This pathway, which is limited to $CD4^+$ T lymphocytes, is termed the indirect pathway of alloantigen recognition. In both pathways, activated $CD4^+$ T lymphocytes secrete various interleukins by which they modulate the effector cells participating in graft rejection (Fig. 2.3).

MHC-molecules are subject to antigen processing and presentation, as shown by sequence analyses of peptides eluted from their binding grooves. The large majority of these peptides derive from self -MHC-molecules [42]. Their presentation increases the sensitivity of naive T lymphocytes to foreign antigens [43]. It is very likely therefore that allo-MHC-molecules are also processed after transplantation and presented as allo-MHC-peptides [44].

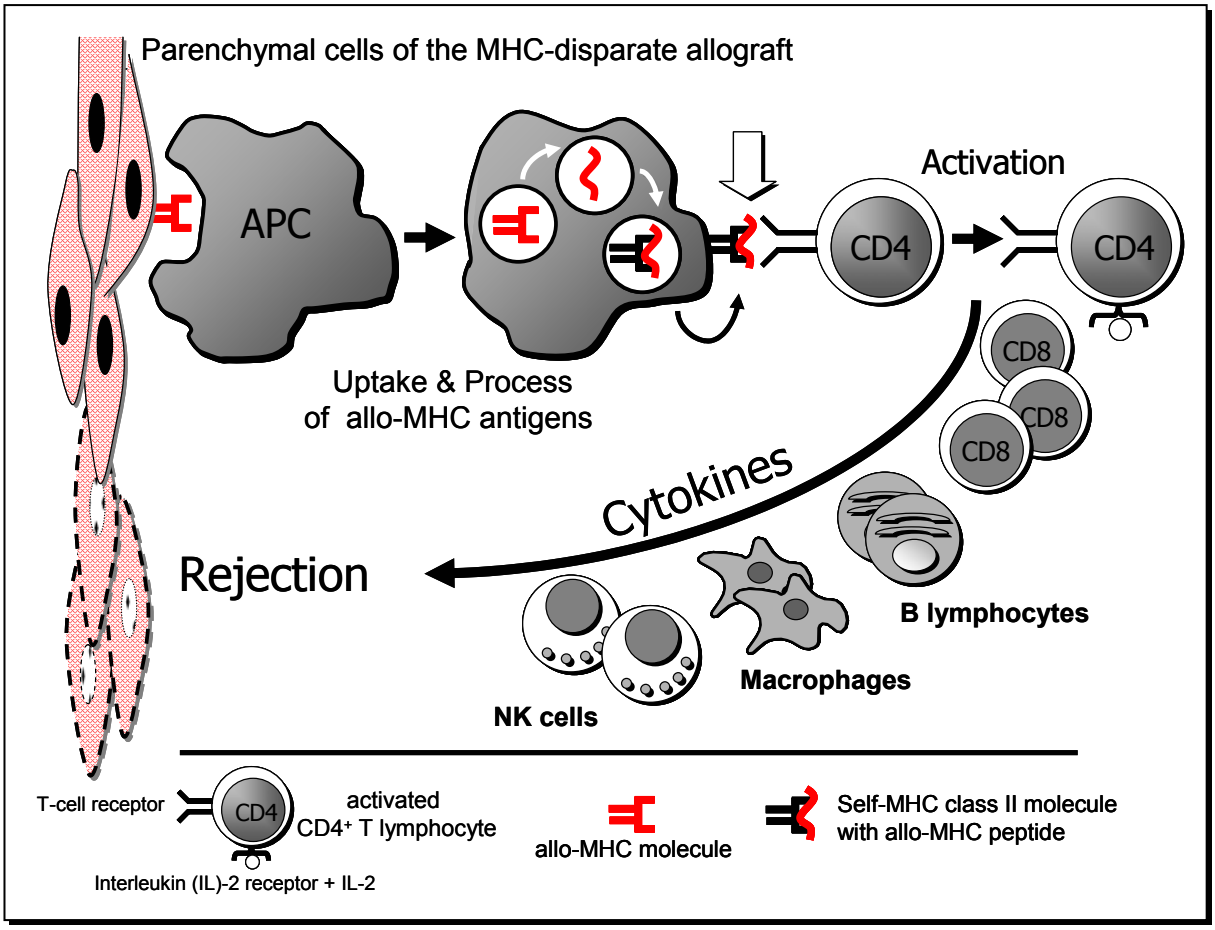

FIGURE 2.3: THE INDIRECT PATHWAY OF ALLOANTIGEN RECOGNITION. Antigen presenting cells (APC) of the host uptake allo-MHC-molecules, process them to allo-MHC-peptides, then present them in self-MHC-class-II molecules on their surface membranes to their own alloreactive $CD4^+$ T lymphocytes [45, 46]. The alloreactive $CD4^+$ T lymphocytes recognizing these allo-MHC-peptides are activated and modulate via their interleukins the effector cells participating in rejection. This pathway, which can also be demonstrated following clinical transplantation [47], appears to be mainly responsible for chronic rejection [48].

2.4 THE IDEAL THERAPY: IMMUNOMODULATIVE STRATEGIES

The serious drawbacks to the immunosuppression concept (Chapter 2.1) underscore the need to develop antigen-specific strategies. The following two strategies for antigenspecific modulation are being investigated:

(1) Modulation with peptide variants or altered peptide ligands [49]. Here the peptide variants prepared from the immunodominant allo-MHC-peptide antigens are used to suppress the activation and proliferation of the $CD4^+$ T lymphocytes

that mediate rejection and/or to promote effector functions such as the production of interleukins so that these cells become effective as regulators [50, 51, 52, 53].

(2) Analysis of the influence of the local alloimmune response on the formation of regulatory $CD4^+$ T lymphocytes. These cells are attractive candidates for the induction of peripheral tolerance. In this connection, the cytokine profile of hepatic $CD4^+$ T lymphocytes following liver transplantation was analyzed for signs of an organ-specific regulator cell population. The liver graft in particular is known to possess unique immunomodulating properties.[4]

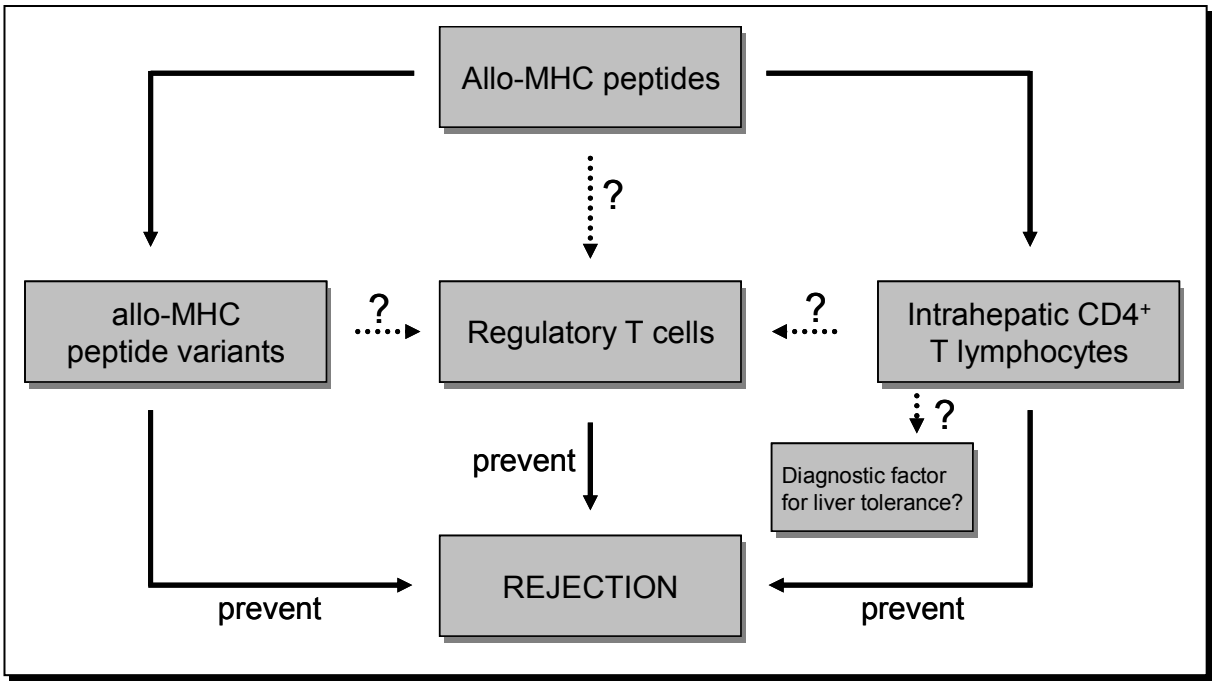

FIGURE 2.4: POSSIBLE ELEMENTS OF THE IDEAL THERAPY. In addition to allo-MHC-peptides, especially allo-MHC-peptide variants, a hepatic $CD4^+$ T-cell population was analyzed for its ability to suppress graft rejection. This cell population, possibly responsible for spontaneous tolerance of liver transplants, could form a basis for the ideal therapy.

[4] Gassel HJ, **Otto C**. Mechanismen der Immuntoleranz nach orthotoper Lebertransplantation. *Transplant Links* 2002; 2: 49-58

2.4.1 IMMUNOMODULATION WITH ALLO-MHC-PEPTIDES

Activation of T lymphocytes requires specific interaction between the $\alpha\beta$ T-cell receptor and the peptide antigens located in the binding grooves of self-MHC-class-II-molecules. Through specific modifications, i.e. through the exchange of individual amino acids, the generated peptide variants change this interaction and thus the activation of T lymphocytes [54], the magnitude of their proliferation and their cytokine profile [55, 56]. Even though the MHC-system is highly polymorphic, the HLA-system for example is know to have more than 1,200 variants [57], it should not be impossible to establish a therapy employing allo-MHC-peptide antigens [58, 59].[5]

Hypothesis 1: Synthetic, immunogenic allo-MHC-peptides, which are responsible for the alloreactivity of a defined donor and recipient combination in certain areas of the allo-MHC-molecules (Fig. 3.2), could form the basis for an antigen-specific therapy following transplantation. Such variants of immunodominant peptide antigens should suppress the activation of alloreactive T lymphocytes and/or modify their cytokine profile in the form of an "immune deviation" [60].

2.4.2 IMMUNOMODULATION WITH REGULATORY $CD4^+$ T LYMPHOCYTES

The recognition of antigens by T lymphocytes generally triggers effective immune response. Under certain conditions however this recognition leads to suppression of the immune response [61, 62]. Investigations into the induction and maintenance of tolerance by regulatory cells are being carried out by among others the team of Waldmann et al. [63]. Their published data on a cellular suppressor mechanism transferred by T lymphocytes [64] has revived the idea of an active form of tolerance induction [65, 66]. Sakaguchi et al. [67] and Groux et al. [68] describe a subtype of $CD4^+$ T lymphocytes that suppresses the immune response both *in vitro* and *in vivo* [69, 70]. Exactly how such cells mediate their regulatory or suppressive properties is not yet known [71]. Since certain functional properties of $CD4^+$ T lymphocytes correlate with a characteristic cytokine profile [72], the underlying mechanism of

[5] Timmermann W, **Otto C**. Chancen durch Vielfalt. Immunmodulation mit HLA-peptidesn. *Z Gastroenterol* 2002; 40: 468 (abstract)

regulatory cells can be interpreted as resulting from specific regulatory interleukins [73, 74].

In 1969 Calne reported that liver grafts in the large animal model swine were sometimes spontaneously accepted (i.e. without immunosuppression) despite a complete MHC incompatibly [75]. The cellular and/or molecular bases of this spontaneous acceptance of liver grafts are still not fully understood [76]. Various strains of inbred rats have proven to be of use in the analysis of this phenomenon [77, 78, 79, 80]. Evidence is growing that the immune response triggered by the liver graft is modulated by the graft itself [81, 82].

Hypothesis 2: The cytokine profile of T lymphocytes with protective immunoregulatory properties exhibit an elevated expression of certain interleukins, including IL-4, IL-10, IL-13 (Th2 cells) [83], and TGF-β (Th3 cells) [84, 85]. The **spontaneous liver graft tolerance** seen in certain rat strains [86] could be based on such a regulatory mechanism.

3 Results & Discussion

3.1 The allo-MHC-peptide-induced Immune response

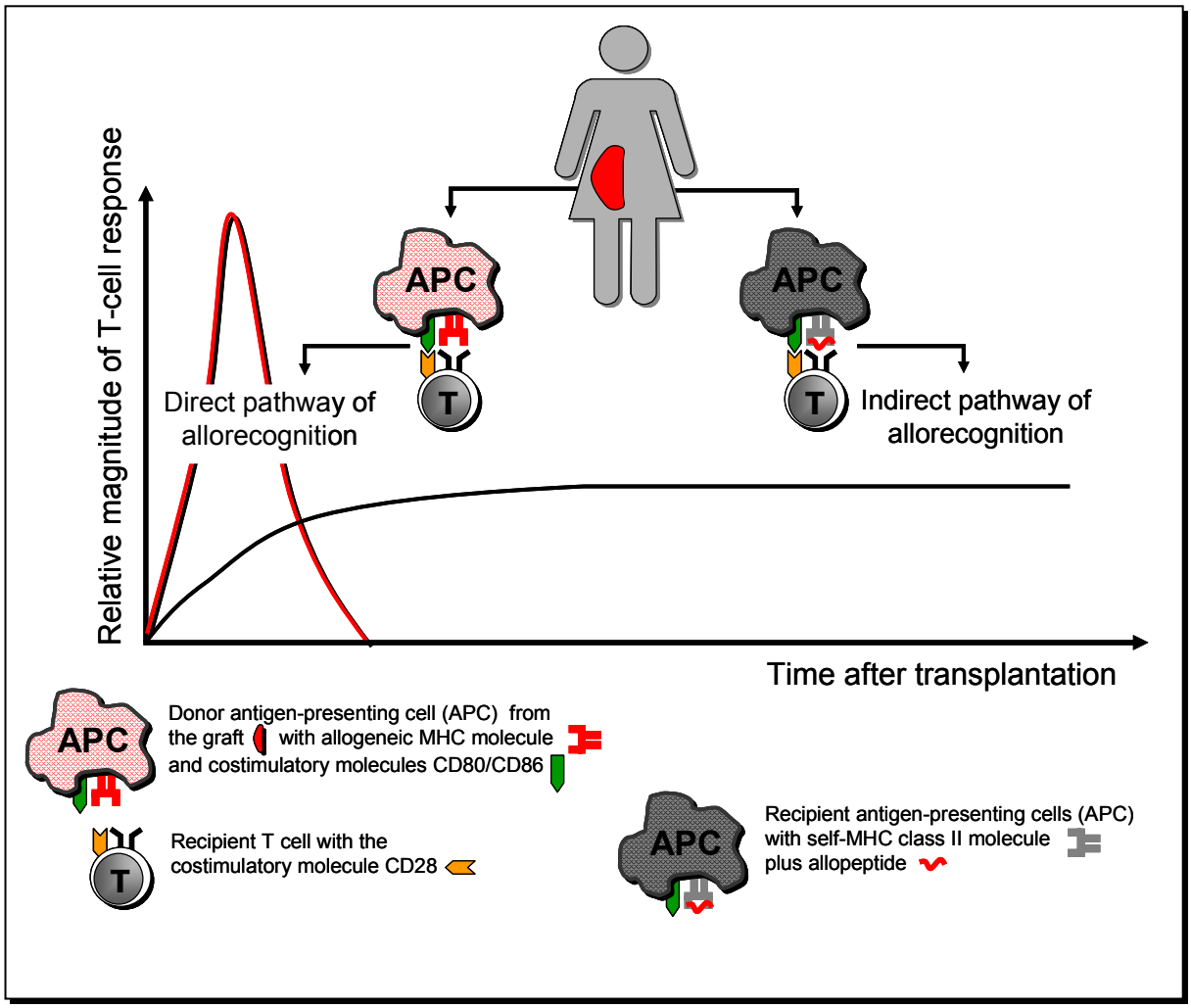

Figure 3.1: The immunological Situation following Organ transplantation. Shown is the relationship between the types and relative strengths of cell activation, and the duration of the resulting alloimmune response leading to rejection.[6, 7] Since the indirect pathway of alloantigen recognition (Fig. 2.3) is dependent on allo-MHC-peptides, modified peptide antigens could be exploited to influence both the activation and function of alloreactive T lymphocytes. The organ graft and *passenger leukocytes* are shown in red, the host MHC-different immune cells in gray.

[6] Timm S, Hamelmann W, **Otto C**, Gassel AM, Etzel M, Ulrichs K, Thiede A, Timmermann W. Influence of donor MHC class I antigen expression on graft survival after rat parathyroid allotransplantation. *Langenbecks Arch Surg* 2001; 386: 430-433

[7] Timmermann W, **Otto C**, Rohde AC, Gasser M, Gassel HJ, Waaga AM, Ulrichs K, Thiede A. Studies about immunogenicity and immunomodulation of rat MHC class II peptides *in vitro* and after orthotopic small bowel transplantation. *Deutsche Gesellschaft für Chirurgie, Forumband* 2000; 29: 263-265

Tolerance, i.e. the immune system's acquired ability to not react to certain antigens, is one of the most impressive phenomena known to immunology [87]. This property of the immune system is of crucial importance in the field of transplantation medicine. In addition to the central tolerance of the thymus based on clonal deletion, the multifarious T lymphocyte reactivity that affects post-thymic processes subsumed under the term peripheral tolerance [88]. This includes not only the "frank" prevention of T-cell activation, but the modulation of mechanisms affecting activation, such as regulatory T lymphocytes. While the current immunosuppression concept can be used to treat acute rejection (Chapter 2.1), its approach is too nonspecific and its long-term application associated with too many side effects. Moreover, continued long-term immunosuppression appears to work against the building of tolerance [89, 90]. One possible explanation for this surprising finding is that such immunosuppressive agents inhibit expression of the growth factor IL-2, which is essential for both the proliferation of alloreactive T lymphocytes and for the retention of regulatory T lymphocytes [91].

As mentioned above, the indirect pathway of alloantigen recognition is based on allo-MHC-peptides (Fig. 2.3). To investigate in detail the effect of such allo-MHC-peptides on the immune system, an repertoire of defined peptides is required. To obtain such peptides, we began by comparing the amino acid sequences of MHC-molecules of a certain combination from the graft donor with those of the recipient. Fig. 3.2 shows this comparison of sequences of the MHC-class-I-molecules for the WF and LEW rat strains. Peptides of the $RT1^u$ locus in the haplotype of the WF rat were synthesized from the areas in which the amino acid sequences of the two rat strain haplotypes differ and investigated for their possible alloreactivity with the haplotype $RT1^l$ of LEW rats. The influence of these peptides on graft rejection was analyzed by giving the LEW rats heterotopic heart transplants from the WF rats after immunization with these $RT1^u$ MHC-class-I-peptides (Fig. 3.4).

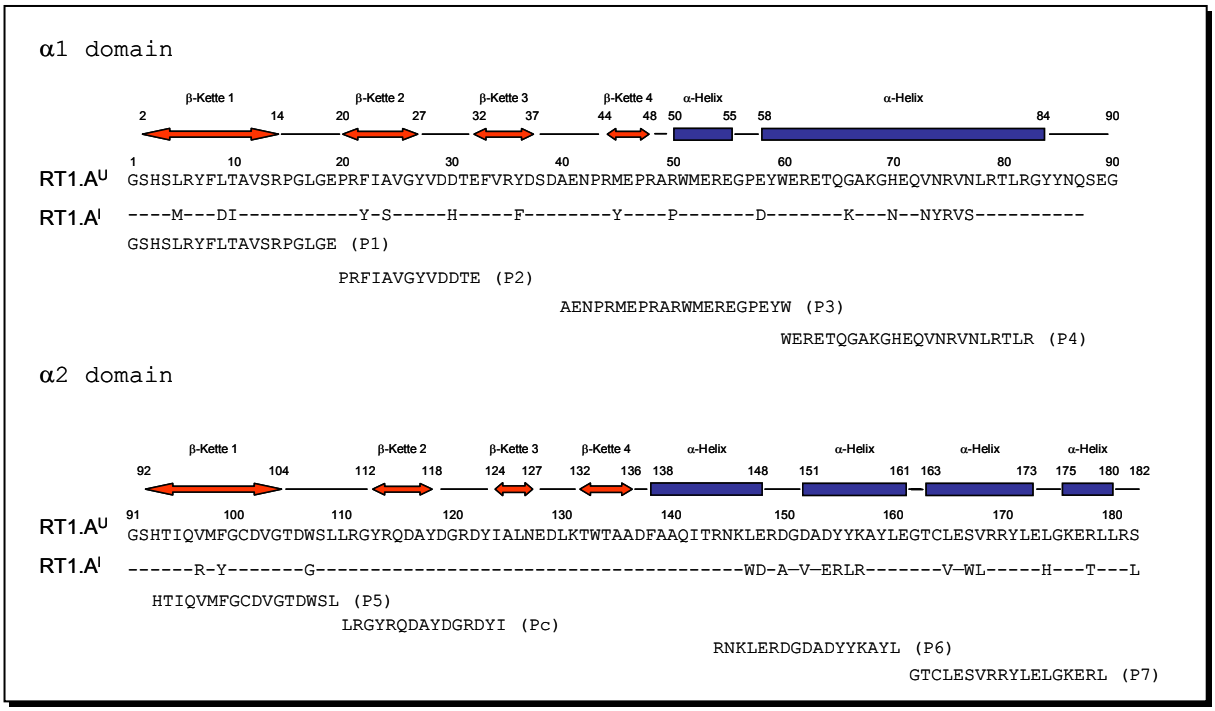

FIGURE 3.2: DEMONSTRATION OF ALLOREACTIVE AREAS IN MHC-CLASS-I-MOLECULES OF HAPLOTYPE RT1^u AS THE BASIS FOR THE PRODUCTION OF SYNTHETIC ALLO-MHC-PEPTIDES. We compared the amino acid sequences of the MHC-class-I-molecules of the two in-bred rat strains Wistar-Furth (WF) (haplotype RT1^u) and Lewis (LEW) (haplotype RT1^l). The two domains α1 and α2 forming the binding groove of the molecule were investigated. The two domains, each comprised of 90 amino acids, differ in 17 amino acids between the two haplotypes RT1^u and RT1^l. The seven RT1^u peptides, P1 to P7, which cover these areas, were tested in LEW rats for their alloreactivity. Two sequences were found to be identical in the area of the peptide Pc. Also shown is the distribution of the ß-chains (red arrows) and α-helices (blue rectangle) within the two domains [92]. The RT1.A^u sequence was published by Joly et al. [93], the RT1.A^l sequence by Salgar et al. [94].

The alloreactivity of these peptides, i.e. their ability to activate alloreactive CD4$^+$ T lymphocytes, was determined in the assay shown in Fig. 3.3. This assay measures T-cell proliferation after a second exposure to the peptide antigen. T lymphocytes not primed by immunization (= first exposure) were not reactivated in the *in vitro* assay under the described culture conditions.

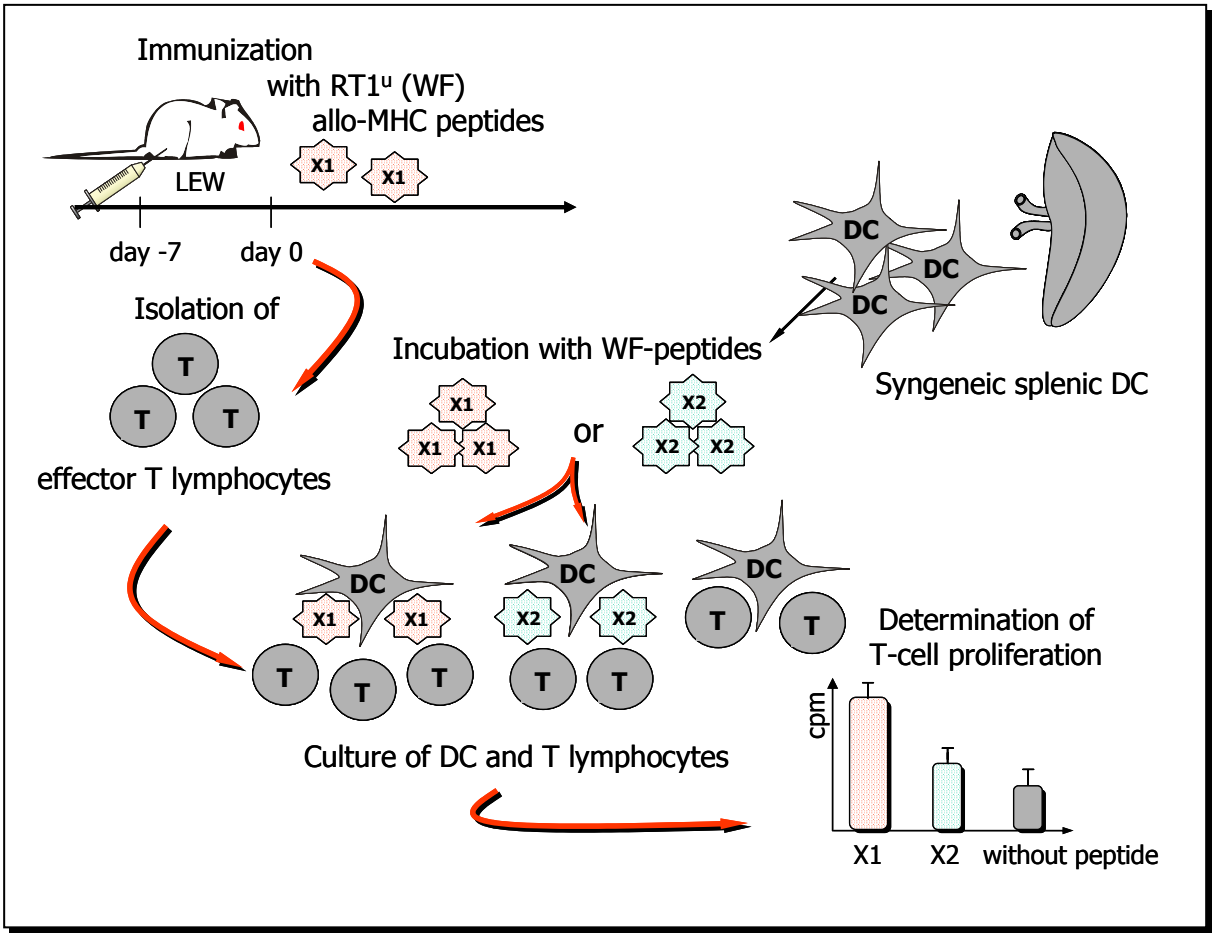

FIGURE 3.3: ASSAY TO MEASURE ALLO-MHC-PEPTIDE-INDUCED T-CELL PROLIFERATION. In a first step RT1^u peptide-specific T lymphocytes were induced by immunization of LEW rats with a WF rat peptide. The subsequent *in vitro* culture, in which the lymphocytes were incubated for three days with peptide-bearing dendritic cells (DC), determined the proliferation induced by the second contact with this peptide. In addition to dendritic cells, other MHC-class-II expressing cells such as B lymphocytes and macrophages can also present synthetic allo-MHC-peptides. [8]

3.1 IMMUNOMODULATION WITH ALLO-MHC-PEPTIDES

Various studies have shown that under certain circumstances allo-MHC-peptides can inhibit or at least delay graft rejection [95, 96, 97, 98]. Their oral application in particular is a highly attractive possibility, even though the mechanism of oral tolerance [99] is not yet sufficiently understood for its successful application in

[8] Timmermann W, Sitaru G, Kottenmeier S, Gassel HJ, Ulrichs K, **Otto C**. Alloantigen specific modulation of the immune response after transplantation using immunodominant peptides. Deutsche Gesellschaft für Chirurgie, Forumband 2003 32: 341-343

clinical practice [100, 101]. It is the contrasting properties of allo-MHC-peptides to act in both an immunostimulatory and immunomodulating manner that makes them especially attractive. They could help to ascertain which factors determine whether exposure to antigens induces T lymphocytes to trigger or suppress an immune response directed against the graft.

The basis for the experiments described in **publication 1** [9] is the long known but not yet fully understood finding that a pretransplantation blood transfusion from donor to recipient has a beneficial effect on later graft function [102]. This strategy, termed transfusion associated immunosuppression (TRIM), was first described by Opelz [103]. Although it lost some of it importance with the introduction of CsA (Chapter 2.2) [104], there is no doubt that such a blood transfusion benefits graft survival [105]. On the other hand, such a donor-specific blood transfusion can also lead to a sensitization of the recipient that accelerates graft rejection [106]. The same effect is produced by allo-MHC-peptides, suggesting they constitute the therapeutically active component of TRIM [107].

Based on these observations we carried out the following experiments with the two immunogenic MHC-class-II peptides RT1.B2 and RT1.D2. As shown in Fig. 3.2 for MHC-class-I peptides, these peptides derived from the $RT1^u$ haplotype represent the allogeneic dominants compared to the $RT1^l$ haplotype (the haplotype of LEW rat) peptides. They activate alloreactive $CD4^+$ T lymphocytes, which in turn induced rejection of vascularized $RT1^u$ grafts in LEW rats. Their possible protective activity on graft function was investigated in two combined approaches, each involving one of the two immunogenic allo-MHC-class-II-peptides and the calcineurin inhibitor CsA. CsA was applied to suppress the activated T lymphocytes. If the recipient animals were sensitized 7 day prior to transplantation with peptide RT1.B2 and treated up to post-transplant day 30 with low-dose CsA, the result was a clear prolongation of graft function. No reactive RT1.B2-specific $CD4^+$ T lymphocytes could be

[9] **Otto C**, Gasser M, Waaga-Gasser AM, Rohde AC, Lenhard M, Jost S, Gassel HJ, Ulrichs K, Timmermann W. Prolongation of small bowel allograft survival with a sequential therapy consisting of a synthetic MHC class II peptide and temporarily low-dose cyclosporine A. *Human Immunol* 2002; 63: 880-887

demonstrated in the RT1.B2-immunized animals. This appears to explain the success of the RT1.B2 combination therapy. The regulator T lymphocytes are the basis of this phenomenon, which is known as "linked suppression" or "linked unresponsiveness" [108, 109]. It is not yet known, however, which allo-MHC-peptides are suitable for protective immunomodulation and which not.[10]

Since the alloimmune response does not appear to be limited to single allo-MHC-peptide antigens, the first step toward a peptide-based immunotherapy needs to explain how to identify candidates suitable for immunomodulation. The goal of **publication 2** [11] therefore was to determine which allo-MHC-class-I-peptide antigens dominate in the alloimmune response, so as then to use them for modification of their amino acid sequence (Tab. 3.1). For this purpose seven different $RT1^u$ peptides were tested for their alloreactivity in LEW rats. These MHC-class-I-peptides represent the areas in the $RT1^u$ haplotype that differ from the $RT1^l$ haplotype, i.e. the amino acids found in this area must be those responsible for the alloreactivity (Fig. 3.2).

Of the seven investigated peptides P1 to P7, immunization with P1 [12] produced the strongest activation. Whereas P1 is by virtue of the strength of the T-cell proliferation it induces the immunodominant peptide, the other six peptides are only slightly (P2-P5) or not at all (P6, P7) immunogenic.

An important question for transplantation immunologists is whether synthetic allo-MHC-peptides, such as the allo-MHC-class-I-peptide P1 also promote the rejection of vascularized organ transplants. Experiments have been preformed to investigate their effect on graft function in a heterotopic heart transplantation model [110] with LEW rats as recipients, WF rats as donors. Immunization with P1 was found to reduce transplant function time in the LEW rats to 4.5 ± 0.5 days versus the 8 days

[10] **Otto C**, Rohde AC, Timmermann W, Waaga AM, Gebert A, Gasser M, Gassel HJ, Thiede A, Ulrichs K. Acceptance of small bowel allografts by indirect allorecognition of donor MHC class II allopeptides. *Transplant Proc* 2001; 33: 431-432

[11] Sitaru AG, Timmermann W, Ulrichs K, **Otto C**. Hierachical immunogenicity of donor MHC class I peptides in allotransplantation. *Human Immunol* 2002; 63: 871-879

[12] Sitaru AG, Timmermann W, Ulrichs K, **Otto C**. Modulation of the T cell response with an MHC class I immunodominant peptide and its analogues. *Immunobiol* 2001; 204: 209 (abstract)

in nonimmunized rats. In addition, transfer of P1-specific T lymphocytes to LEW rats reduced the transplant function time of heterotopic heart transplants to 5.3 ± 0.5 days. A peptide that is not immunogenic in proliferation assays (Fig. 3.3) also does not affect the time of rejection.

The presentation of allo-MHC-peptides by MHC class-II molecules is a key event in the indirect T-cell response against alloantigens of the transplant. The characterization of these synthetic allo-MHC-class-I peptide antigens supports the view that their allogenicity exhibits a hierarchical distribution [111], a phenomenon also observed in clinical practice [112, 113]. This means that the alloreactive T lymphocytes do not recognize all antigen determinants identified by sequence comparison. This important observation appears to diminish the number of MHC peptide antigens to a few candidate peptides and supports the optimism regarding the development of an antigen specific immunotherapy. When these peptides are tested in another rat strain the distribution of the alloreactivity also changes. This shows that any possible antigen-specific immunotherapy must be tailored to the particular donor-recipient combination.

3.2 IMMUNOMODULATION WITH ALLO-MHC-PEPTIDE VARIANTS [13]

Our goal is to suppress the activation and proliferation of alloreactive T lymphocytes using variants made from the immunodominant allo-MHC peptide antigens. It would be extremely attractive to modulate certain effector functions, such as the production of interleukins, in such a manner that these cells become active as regulators. We investigated the effect different variants derived from the immunodominant peptide P1 had on the activation of alloreactive T lymphocytes and graft function. For this purpose the three divergent amino acids in the MHC-class-I molecules WF rat were sequentially replaced by those located at the same position in the MHC-class-I molecules of LEW rat (Table 3.1).

Table 3.1. PRODUCTION OF ALLO-MHC-CLASS-I-PEPTIDE VARIANTS. The variants A1.1 to A1.6 are derived from peptide P1, which is identical to the corresponding sequence of the $RT1^u$ haplotype (cf. also Fig. 3.2). In the first 19 amino acids the two haplotypes $RT1^u$ and $RT1^l$ differ at positions 5, 9 and 10. The six P1 variants originate from sequential amino acid exchanges between the two haplotypes (unpublished data). Positions 5, 9 and 10 represent, since they are the only differences between the $RT1^u$ and $RT1^l$ haplotype in this area in MHC-class-I molecule, the recognition or core sequence for P1-specific T lymphocytes.

Peptide	**Amino acid Sequence**																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
$RT1A^u$	G	S	H	S	**L**	R	Y	F	**L**	**T**	A	V	S	R	P	G	L	G	E
P1	G	S	H	S	**L**	R	Y	F	**L**	**T**	A	V	S	R	P	G	L	G	E
A1.1	-	-	-	-	**M**	-	-	-	**L**	**T**	-	-	-	-	-	-	-	-	-
A1.2	-	-	-	-	**L**	-	-	-	**D**	**T**	-	-	-	-	-	-	-	-	-
A1.3	-	-	-	-	**L**	-	-	-	**L**	**I**	-	-	-	-	-	-	-	-	-
A1.4	-	-	-	-	**M**	-	-	-	**D**	**T**	-	-	-	-	-	-	-	-	-
A1.5	-	-	-	-	**L**	-		-	**D**	**I**	-	-	-	-	-	-	-	-	-
A1.6	-	-	-	-	**M**	-	-	-	**L**	**I**	-	-	-	-	-	-	-	-	-
$RT1A^l$	G	S	H	S	**M**	R	Y	F	**D**	**I**	A	V	S	R	P	G	L	G	E

[13] Sitaru AG, Timmermann W, Ulrichs K, **Otto C**. Allogeneic core amino acids of immunodominant allopeptide are important for MHC binding and TCR recognition. *Human Immunol* 2004; 65: 817-825

Our results show that with the exception of A1.5 all P1 variants of P1-specific T lymphocytes were recognized. The slight immunogenicity of A1.5 for P1-specific T lymphocytes was also confirmed in the transplantation model (Fig. 3.4). The "normal" rejection time for this strain combination was 8 days. The same time point was also observed after sensitization with the A1.5 variant. If the recipient was immunized subcutaneously with a combination of A1.5 and P1 at the same molar concentrations prior to transplantation, the sensitization effect of P1 was not observed and the transplant was rejected on day 8. But if P1 and A1.5 were applied separately, then A1.5 had no protective effect and the graft was rejected on day 5, the same as after immunization with P1 (Fig. 3.4).

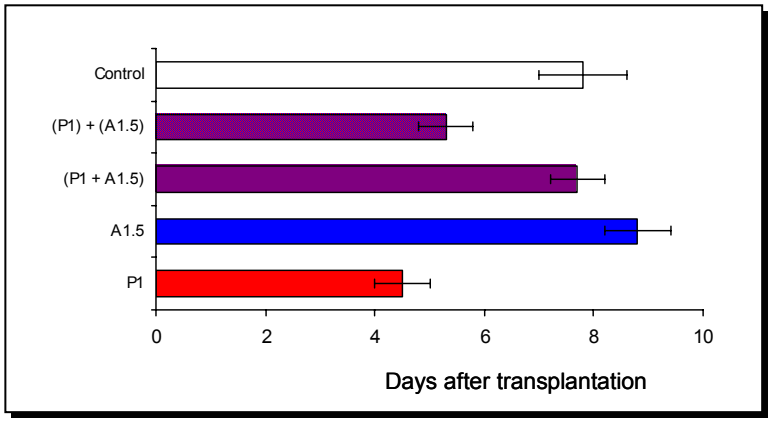

FIGURE 3.4: THE *IN VIVO* EFFECT OF A1.5 ON TRANSPLANT FUNCTION. The P1 peptide variant A1.5 delays the immunodominant peptide P1-induced accelerated transplant rejection when it is applied together with the P1 peptide (P1 + A1.5), but not when the two peptides are applied separately [(P1) + (A1.5)]. The results are shown as mean ± standard deviation of n = 4 animals.

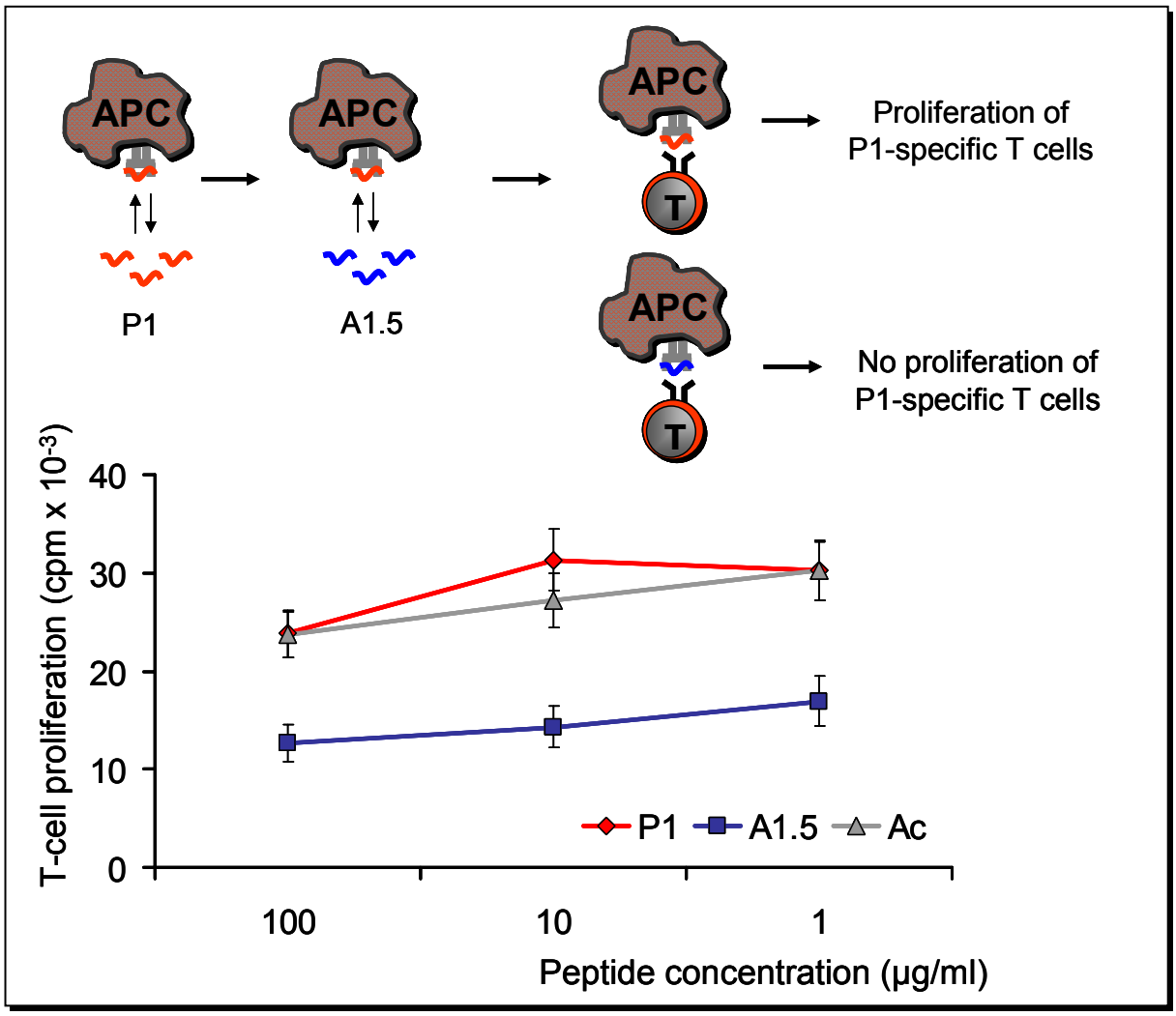

FIGURE 3.5: PRINCIPLE AND RESULTS OF THE MHC-INHIBITION ASSAY. The measured proliferation of the P1-specific cells shows whether the P1 peptide continues to be reside within the binding groove formed by the MHC-class-II-proteins or has been displaced by the A1.5 variant. The results indicate that the A1.5 variant did indeed displace the P1 antigens. Since A1.5 is a suboptimal antigen for P1-specific T-Lymphocytes, this results in diminished proliferation. The control peptide Ac (a peptide representing and area of the MHC-class-I molecule in which both haplotypes are identical) does not possess this property.

The exchange of individual amino acids in immunogenic peptides can influence both recognition via the T-cell receptor and binding to the MHC-class-II molecule. We investigated whether the A1.5 variant can displace already bound P1-peptides from the MHC-class-II-binding groove. For this purpose APC were first loaded with the immunodominant peptide P1 and then incubated with the A1.5 peptide variant at the

same molar concentration. Should A1.5 exhibit greater binding affinity for the $RT1^l$ MHC-class-II-molecule, then P1 will be displaced from the binding groove and the subsequent incubation with P1-specific T lymphocytes lead to diminished proliferation. The results of the MHC-inhibition assay support this interpretation (Fig. 3.5).[14, 15]

In the indirect pathway of alloantigen recognition a highly diverse array of allo-MHC-peptides with defined allogeneic determinants are presented to the alloreactive $CD4^+$ T lymphocytes. This pathway is therefore especially well-suited for the introducing altered synthetic peptides into the immune system. Whereas the identification of immunodominant allo-MHC-peptides is already possible for certain donor–recipient combinations, variants with immunomodulating properties can not yet be produced. The molecular knowledge for ascertaining whether allo-MHC-peptides possess immunomodulating and/or immunoactivating properties, a prerequisite for clinically feasible development of such procedures, is still lacking [114, 115]. It must especially be determined whether the immunomodulating properties have a definite structural pattern in these peptides. Knowledge of the structure of the T-cell receptors of tolerogenic T lymphocytes could be of help in recognizing such tolerogenic peptide structures, i.e. knowledge as to which amino acids must be located at which positions in the peptide so that interaction with the tolerogenic T-cell receptor can occur. This knowledge can be used to direct the computer-aided search for similar structures in peptide databanks. The procedure presented here (Tab. 3.1) for production of variants could be an alternative.

[14] **Otto C**, Sitaru G, Ulrichs K, Timmermann W. Design of peptide analogues as a strategy to modulate the alloresponse of peptide-specific T cells. *Transplantation* [Suppl] 2002; 74: 271 (abstract)

[15] Sitaru G, Timmermann W, Ulrichs K, **Otto C**. Immunodominant peptide analogues as a strategy to modulate the alloimmune response. *Immunobiol* 2002; 206: 283 (abstract)

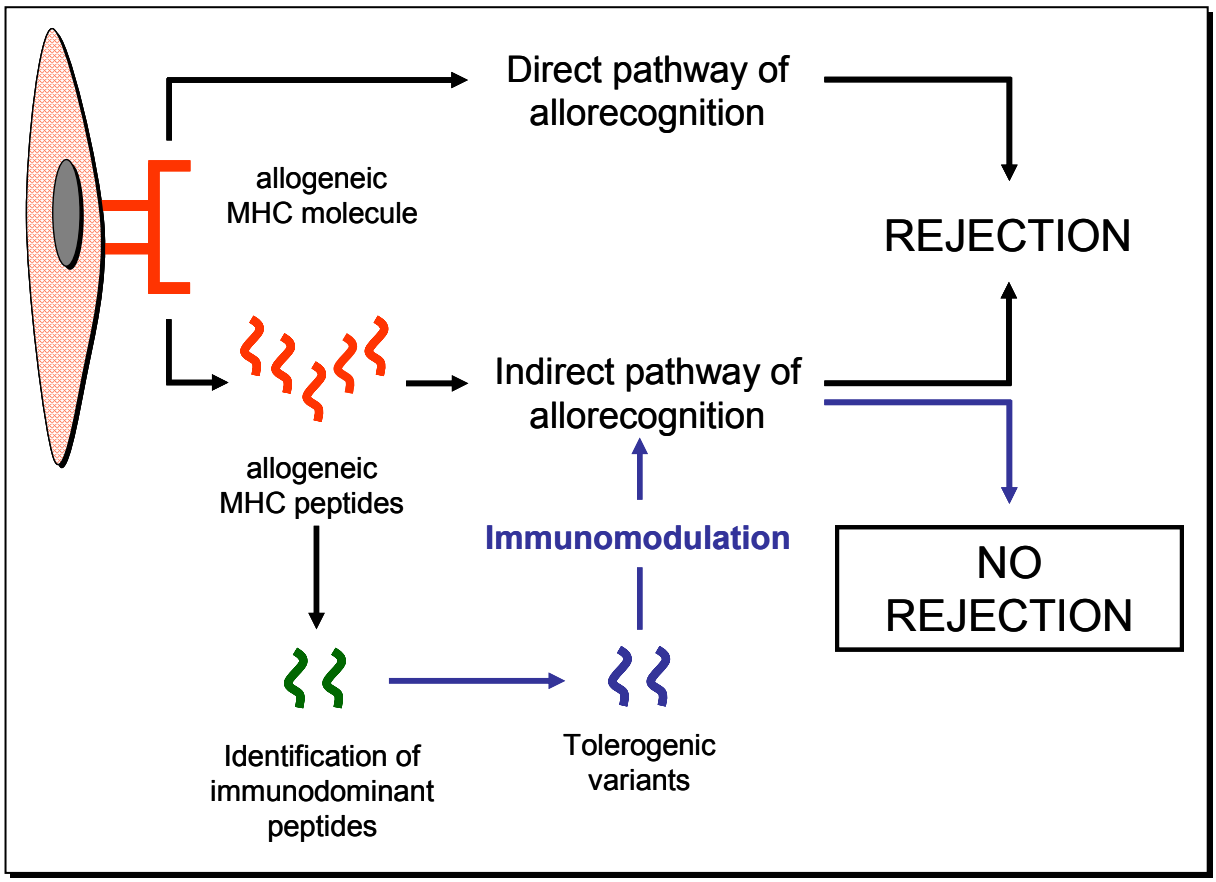

FIGURE 3.6: IMMUNOMODULATING PEPTIDE VARIANTS AS THE BASIS FOR THE IDEAL THERAPY FOR ANTIGEN-SPECIFIC SUPPRESSION OF TRANSPLANT REJECTION. The problem will not be the large-scale production of such variants, using e.g. *"phage display"* banks, but their biological testing with *"high-throughput-screening"*. The alternative would be to test only a few highly promising candidates in the assay presented here (Fig. 3.3). A cell of the graft with alloantigens (either allo-MHC-class-I or class-II-molecules) is shown in red.

3.4 IMMUNOMODULATION WITH REGULATORY CD4$^+$ T LYMPHOCYTES

Among the possible mediators of peripheral tolerance, regulatory T lymphocytes stand at the center of interest. It is conceivable that regulatory T lymphocytes generated in vivo or in vitro could be used to suppress graft rejection.

Two major problems currently occupy transplantation immunologists: One is the difficulty of successfully applying the tolerance-inducing concept in clinical practice, the other is the difficulty of establishing systems to reliably test for tolerance. At present no or only scant information is available on whether liver transplant

recipients can discontinue immunosuppression when graft tolerance has become established in them. No physician would dare stop immunosuppression or reduce doses without definite indications of stable tolerance. The main criterion at present for selecting patients for dosage reduction is the time of rejection-free episodes following transplantation (die Zeit rejectionsfreier Episoden nach Transplantation) [116, 117]. It is uncertain however whether this is sufficient as a selection criterion. A reliable marker of tolerance would represent a giant step forward. It is also unclear just what would form such a marker would take, whether it would be a certain cell population, a certain interleukin pattern, an intracellular component such as an enzyme in the signal chain, or a transcription factor. It is in this context, i.e. the search for a marker of tolerance, that the following experiments are to be regarded.

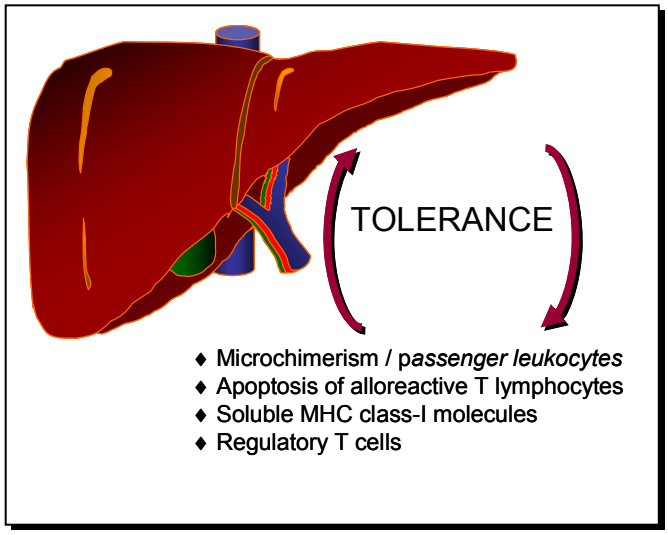

FIGURE 3.7: SURVEY OF THE POSSIBLE MECHANISMS OF SPONTANEOUS TOLERANCE OF LIVER TRANSPLANTS [118, 119, 120].

3.4.1 IMMUNOBIOLOGICAL PROPERTIES OF LIVER TRANSPLANTS

The investigations in **publication 3** [16] focused on whether in immunosuppressive combination therapies monoclonal antibodies can compensate for the diminished immunosuppressive potency brought on by the reduction of dosage. Such studies are important insofar as they underpin our working hypothesis that certain hepatic cell

[16] Gassel HJ, **Otto C**, Gassel AM, Meyer D, Steger U, Timmermann W, Ulrichs K, Thiede A. Tolerance of rat liver allografts induced by short-term selective immunosuppression combining monoclonal antibodies directed against CD25 and CD54 with subtherapeutic cyclosporine. *Transplantation* 2000; 69: 1058-1067

populations may be associated with the tolerance of liver transplants.[17] These data are also highly interesting since combination therapy with CsA and the two antibodies NDS-61 (blockade of the IL-2 receptor) and 1A29 (blockade of cell-cell interaction) induces long-term acceptance of liver grafts based on antigen-specific tolerance. All of the agents in this selective immunosuppression were applied at minimal effective doses [121]. The blockade of cell adhesion by 1A29 hampers the interaction between the alloreactive T lymphocytes and the APC. CsA suppresses the transcription of IL-2 mRNA in activated T lymphocytes, whereas NDS-61 blocks the formation of IL-2 on the IL-2 receptor. This immunosuppressive protocol thus acts mainly in the early phase of T-cell activation. The extent to which this protocol contributes to the propagation of regulatory T lymphocytes can only speculated on at this time. It is known that in order to survive CD25+ regulatory T lymphocytes require -- in addition to the T-cell receptor-mediated signal -- exogenous IL-2 (page 15). The CD25+ cells are, as we and others have shown, also a component of the lymph node population in rat.[18]

Publication 4 [19] presents the hepatic lymphocyte population in tolerated liver transplants. The immunobiological properties of hepatic leukocytes, especially the T lymphocytes, appear to differ markedly from those of leukocytes in other organs. The liver possesses, for example, dendritic cells and a very large macrophage population. The necessity of not inducing an immune response to the harmless constituents of nutrients explains why the local presentation of antigens usually does not lead to the propagation of activated T lymphocytes. The survival of activated T lymphocytes is limited by both "activation-induced cell death" and "death by neglect" [122]. The acceptance and tolerance of liver transplants are attributed in part to

[17] **Otto C**, Gassel H-J. Look insight: Die Leber als immunologisches Organ. *Z Gastroenterol* 2002; 40: 468 (abstract)

[18] **Otto C**, Kuckein O, Ulrichs K. Characterization of rat $CD4^+$ $CD25^+$ T regulator cells. *Immunobiol* 2003; 208: 254

[19] Gassel HJ, **Otto C**, Klein I, Steger U, Meyer D, Gassel AM, Ulrichs K, Thiede A. Persistence of stable intragraft cell chimerism in rat liver allografts after drug-induced tolerance. *Transplantation* 2001; 71: 1848-1852

such effects [123].[20] Studies on local cytokine patterns also point to the presence of regulatory T lymphocytes [124].

A further discovery of recent years is that dendritic cells, which were originally regarded as potent activators of an adaptive immune response, are also effective inhibitors of an immune response [125] via interleukins or cell-cell-contacts [126]. Hepatic DC also possess tolerogenic properties [127]. Some hepatic subpopulations are characterized by a reduced expression of costimulatory molecules [128]. We could thus show that tolerated liver transplants possess hepatic dendritic cells with diminished expression of the costimulatory molecules CD80 and CD86.[21] **Publication 5**[22] describes the long-term presence of intrahepatic DC in tolerated liver transplants.

3.4.2 Demonstration of Hepatic CD45RC^{neg} T lymphocytes

Publication 6[23] investigates whether in certain situations after transplantation the formation of Th2 cells, including possibly regulatory T lymphocytes, is promoted [129]. Our own data on possible tolerance-inducing hepatic populations (publications 3 to 5) suggest that the alloimmune response especially in the liver transplant leads to the formation of regulatory T lymphocytes.[24]

$CD4^+$ T lymphocytes can be further differentiated according to their expression of certain isotypes of the CD45-molecules ("leukocyte common antigen") [130]. In rat the OX22 antibody is a marker of the high molecular-weight isoform of CD45RC

[20] Gassel HJ, **Otto C**, Klein I, Meyer D, Timmermann W, Ulrichs K, Thiede A. Analysis of cellular events in hepatic allografts: Donor progenitors induced intragraft chimerism. *Transpl Int* 2000; 13 [Suppl 1]: 465-470

[21] **Otto C**, Öhrlein E, Meyer D, Timmermann W, Gassel HJ, Thiede A, Ulrichs K. Detection of dendritic cells with downregulated CD80/CD86, but normal MHC class II expression after rat liver transplantation. *Transplant Proc* 2001; 33: 442-444

[22] Meyer D, Löffeler S, **Otto C**, Czub S, Gassel HJ, Timmermann W, Thiede A, Ulrichs K. Donor-derived alloantigen-presenting cells persist in the liver allograft during tolerance induction. *Transplant Int* 2000; 13: 12-20

[23] **Otto C**, Kauczoc J, Martens M, Steger U, Möller I, Meyer D, Timmermann W, Ulrichs K, Gassel HJ. Mechanisms of tolerance induction after rat liver transplantation: Intrahepatic $CD4^+$ T cells produce different cytokines during rejection and tolerance in response to stimulation. *J Gastrointest Surg* 2002; 6: 455-463

[24] Gassel HJ, **Otto C**. Mechanismen der Immuntoleranz nach orthotoper Lebertransplantation. *Transplant Links* 2002; 2: 49-58

[131]. Two distinct $CD4^+$ subpopulations are distinguished: $CD45RC^{pos}$ cells, which represent the naïve phenotype, and $CD45RC^{neg}$ cells. These are activated cells or so-called "memory cells". When stimulated the $CD4^+$ $CD45RC^{pos}$ T lymphocytes produce more IL-2 and IFN-γ than IL-4, the $CD4^+$ $CD45RC^{neg}$ T lymphocytes more IL-4 than IL-2 and IFN-γ [132, 133].

The $CD4^+$ $CD45RC^{neg}$ T lymphocytes in particular appear to have immunoregulatory properties, some data indicating that the organs from which they are isolated also have an influence on the properties of these cells. Thus $CD45RC^{neg}$ T lymphocytes isolated from spontaneously tolerated liver transplants differ from those isolated from the spleen of the same animal. When activated in vitro $CD4^+$ $CD45RC^{neg}$ T lymphocytes isolated from tolerated liver transplants produce the immunoregulatory interleukin IL-13 at both the mRNA and protein level[25]. IL-13 production could be demonstrated from day 30 to day 100 after transplantation, when the study ended. This property is not possessed by the other hepatic $CD4^+$ subpopulation with the $CD45RC^{pos}$ phenotype as well as $CD4^+$ $CD45RC^{neg}$ T lymphocytes of the spleen.[26]

It is not known whether the Th2 interleukin IL-13 performs an immunomodulating function within the liver by suppressing macrophage-induced inflammatory processes [134]. IL-13 could also be the sought after marker for both hepatic regulatory T lymphocytes and stable transplant tolerance. It remains for future studies to elucidate the regulatory function of these cells.

[25] Gassel HJ, Kauczok J, Martens N, Steger U, Timmermann W, Ulrichs K, **Otto C**. Tolerance induction following orthotopic rat liver transplantation: Cytokine production by $CD4^+$ T cells determines the immunological response. *Transplant Proc* 2002; 34: 1429-1430

[26] **Otto C**, Schmitz P et al. Publication in preparation

The liver's unique ability to down regulate local immune responses could also be used to more effectively induce the so-called "linked unresponsiveness" mediated by allo-MHC-peptides and their variants. One possibility is the targeted vesicle-mediated transport of these peptides into the liver. Or the genetic information of these allo-MHC peptides could be permanently or temporarily introduced into and subsequently expressed by the hepatocytes (Fig. 3.8).

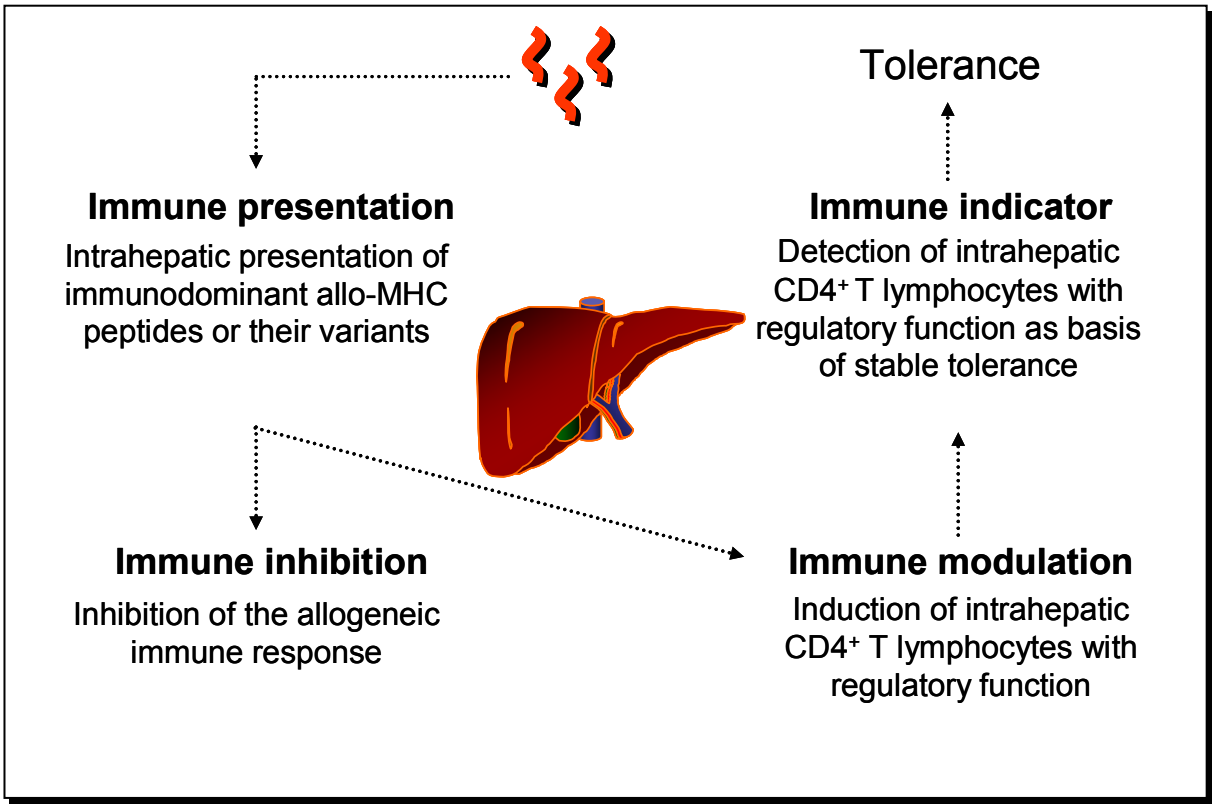

FIGURE 3.8: STRATEGY FOR INCREASING THE MODULATION POTENTIAL OF MHC-CLASS-I PEPTIDES OR THEIR VARIANTS BY APPLICATION IN THE LIVER. This approach may promote the formation of hepatic regulatory cells. It is based on the (still hypothetical) connection between allo-MHC-peptides and their potential to induce regulatory T lymphocytes as shown in Fig. 2.4. Moreover, these cells and/or the interleukin IL-13 they secrete could serve as biological markers of an altered immunological situation and thus as the sought after marker of tolerance. The allo-MHC-class-I-peptides and/or their variants are shown in red.

4. PROSPECTS/OUTLOOK

The two experimental approaches for modulation of the immune system with allo-MHC-peptide variants and regulatory $CD4^+$ T lymphocytes presented here are clinically best suited for living donations (kidney or partial liver donation). Due to the stagnating number of brain-dead organ donors, living donations represent an increasingly important alternative. Since the operation can be planned in advance, there should be sufficient time to carry out the necessary examinations and analyses needed for an immunomodulating strategy. Even if the five-year survival rate for kidney transplants from living donors is very high at 80%, the expenses an immunomodulating procedure entails is certainly justified if it brings one closer to the long-term goal of 100% transplant function.

Although both ideal therapy concepts presented here, immunomodulation with allo-MHC-peptide variants and regulatory $CD4^+$ T lymphocytes, are still in the experimental phase, strategies for their clinical application can already be planned (Fig. 4.1). Whereas the immune response induced by the direct pathway of alloantigen recognition can be inhibited by short-term immunosuppression, the indirect pathway of alloantigen recognition allows Immunomodulative approaches using allo-MHC peptide variants. It is conceivable that these peptides can be used to inhibit the immune response, modulate T-cell activation, or -- and this would be and extremely attractive strategy -- induce the formation of regulator cells. The success of such a therapy depends in part on the extent to which the phenomenon of *"epitope spreading"*, that is the expansion of the epitope repertoire, can be controlled in the late phase after transplantation. In the early phase, the alloimmune response is limited to a few dominant epitopes, whereas in the late phase further antigens are involved that can be recognized by alloreactive T lymphocytes [135, 136].

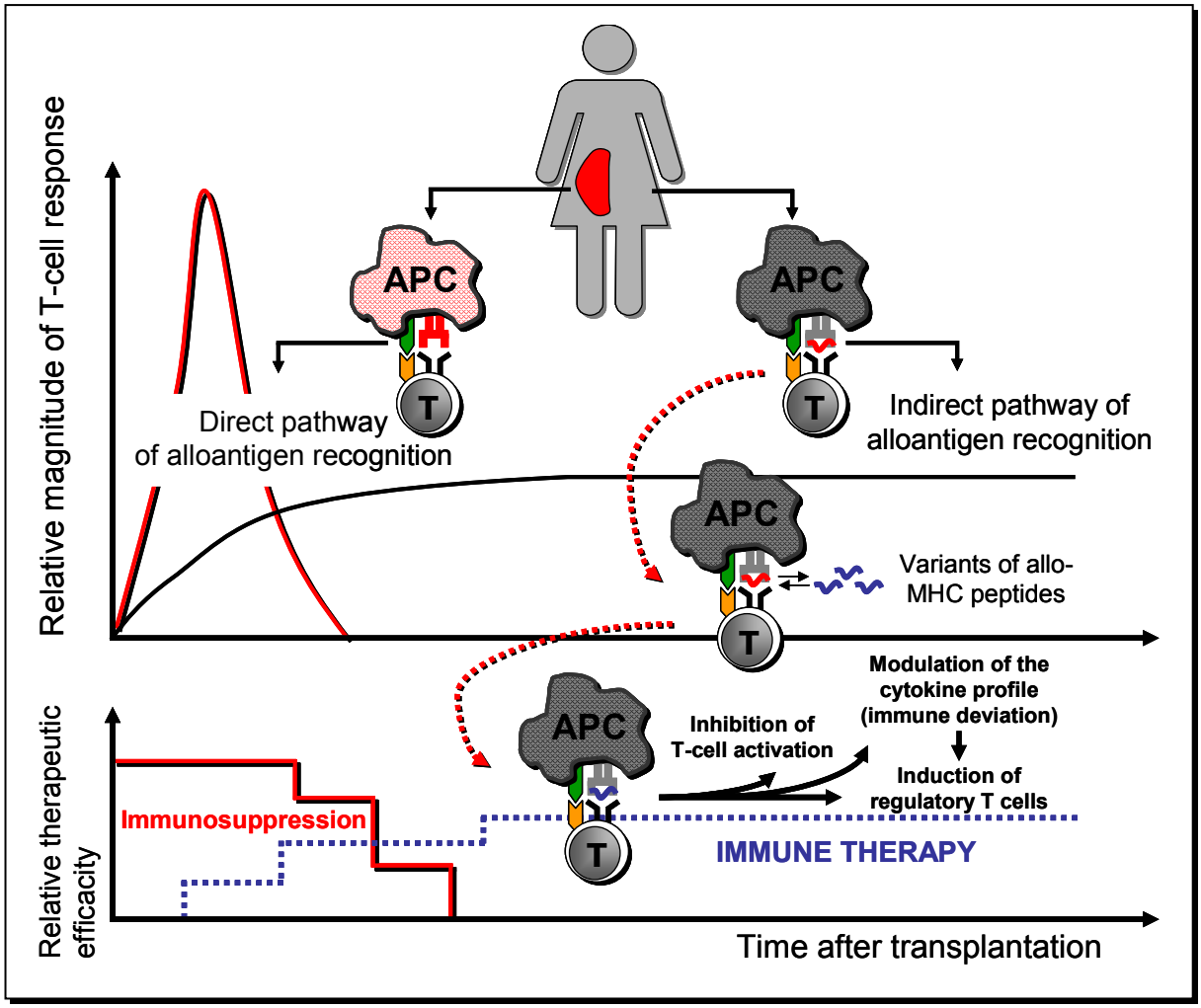

FIGURE 4.1: POSSIBLE CLINICAL APPLICATIONS OF THE IDEAL THERAPY. This procedure would use a combination of short-term conventional immunosuppression and an antigen-specific immunotherapy. The organ transplant and the *passenger leukocytes* are shown in red, the immune cells of the host in gray.

The antigen-specific modulation of the T-cell response after transplantation probably also promotes the cellular and molecular mechanisms leading to tolerance, in order to also clinically realize the ultimate goal of transplantation research, the induction of transplant-specific tolerance. That this continues to be of undiminished relevance is underscored by the founding of the international Immune Tolerance Network for initiating and coordinating promising clinical studies on tolerance induction as well as on validating test systems for confirming tolerance [137]. Here, molecular-immunological markers for reliable demonstration of tolerance are of supreme important. The currently available *in vitro* assays are not always capable of

demonstrating the phase of stable tolerance [138, 139, 140]. One reason for this is that the underlying mechanisms that determine when an activated T lymphocyte becomes alloreactive or tolerant are not fully understood. This then is a basic prerequisite for successful establishment of such assays.

The phenomenon of clonal deletion is an extremely important mechanism for limiting the formation of autoreactive T lymphocytes [141, 142]. It is also essential for the induction of transplant-specific tolerance, although so-called active mechanisms are currently preferred, such as the presence of regulatory T lymphocytes [143]. This also means that an altered immunological situation (= tolerance) after transplantation could be demonstrated by the presence of such cells and/or of the interleukins they secrete. This new way of approaching the problem could contribute to the solving of one of the basic problems in transplantation, the lack of markers of tolerance.

Recent data point to a genetic predisposition affecting whether or not a recipient rejects a transplant under immunosuppression [144]. The Human Genome Project could provide invaluable data for such predictions in particular, but also for the development of individualized immunosuppression.

Transplantation is currently the only therapy for irreversibly damaged organs. Although its success is due mainly to the immunosuppression concept, the latter's severe shortcomings demand the development of new strategies. The experimental approaches for antigen-specific immunomodulation presented here could form the basis for both an ideal therapy and new diagnostic concepts.

5. LITERATURE

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