MHC Restriction and Allogeneic Immune Response

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The discovery of the phenomenon of MHC restriction provided a key for understanding how T lymphocytes recognize the antigens presented on pathogenic bacteria, viruses and tumor cells. The MHC molecules are natural ligands for endogenous and exogenous peptides whose complexes are recognized by T cell receptors that specifically interact with the MHC molecule and the peptide. The interpretation widely accepted until recent time stated that MHC restriction is a consequence of "adaptive differentiation" in the thymus, and during this differentiation the forming repertoire of T cells "learns" the low affinity interaction with self MHC molecules through positive selection. This theory was based on the experimental data on radiation chimerae with the immune system restored by grafting of the bone marrow from semiallogeneic animals. However, R. Zinkernagel and colleagues have countered this viewpoint by uncovering an artifact in the above experiments, namely, incomplete removal of recipient's T cell precursors and, therefore, "non-equal" starting conditions compared with the transplanted donor cells. The adaptive differentiation failed to unambiguously explain the direct allogeneic and allorestricted recognition phenomena. Novel data obtained from the selection of T cell repertoire in TCR transgenic animals, the expression of individual MHC/peptide complexes and recipients of xenogenic thymus provide evidence for high ability to adapt to microenvironment and low specificity of positive selection. These facts lay the foundation for an alternative interpretation of MHC restriction. In particular, this phenomenon is likely to be explained by the specificity of the pool of effector cells activated by primary immunization. The detailed molecular mechanisms of this phenomenon have been described by H.G.Rammensee's group who demonstrated differential primary structures of the peptides that bind various allelic forms of MHC molecules. The T lymphocyte repertoire is formed in the thymus as a result of random rearrangement of germinal sequences of TCR gene fragments and other processes that bring about the diversity of TCRs. As shown by D.Raulet and co-workers, such pre-selected repertoire of T lymphocytes is inherently capable of reacting with different allelic forms of MHC molecules. In contrast to germinal sequences of TCR fragments, the MHC molecules are characterized by a significant interspecies polymorphism. Therefore, negative and positive selection are aimed at the adaptation of pre-selected repertoire to the specific microenvironment of certain individual. The overall goal of adaptation is the elimination of autoreactive clones and sparing a broad spectrum of specificity to potential pathogens. From this viewpoint the selection in the thymus can be considered as a life-long allogeneic reaction of pre-selected repertoire to self MHC molecules; the responses of the mature T cell repertoire to individual peptides are just the examples of transplantation reactions. I believe that this interpretation can reconcile some discrepancies in modern views on MHC restriction and unveil the origins and biological meaningfulness of allogeneic reactions, the problem unprecedentally difficult for its pioneers.

1. Discovery of MHC restriction.

The dependence of the immune response and immunorecognition on MHC class I molecules has been demonstrated by Rolf Zinkernagel and Peter Doherty on the model of infection with lymphocytic choriomeningitis virus (LCMV) [1]. As R. Zinkernagel stated in his Nobel lecture, the basis for MHC restriction hypothesis was laid with development of the test for cytotoxicity of T cells to LCMV infected cells. Initially this test was used to study whether there is a correlation between T cellular response to LCMV and the gravity of choriomeningitis. Doherty

took an aliquote of murine cerebrospinal liquor whereas R. Zinkernagel measured the cytotoxicity of cells in this sample using his novel Cr^{51} release test. The authors found that T cells that specifically lysed the infected targets can be detected in the liquor of infected immunocompetent mice but not in *nude* mice; these cells can be a major factor in pathogenesis of lethal choriomeningitis. The article containng these findings appeared in J.Exp.Med., 1973, and in the same year M. Oldstone and H. McDevitt published their results on differential sensitivity to intracerebral LCMV infection of mouse strains that vary in MHC gene repertoire [2, 3]. In turn, Zinkernagel and Doherty set out to do additional experiments with inbred mice and their hybrids taht were available at John Cartin School of Medical Research. Although the intracerebral infection was equally lethal for all strains, virus specific CTLs in the spleen were seldom. This fact can be interpreted by either an inadequate test or by lack of the role of CTLs in the pathogenesis of lethal choriomenigitis. The former suggestion appeared to be true: the L929 cell line used by Zinkernagel and Doherty, originated from murine C3H ($H\text{-}2^k$) line whose MHC molecules are identical to those in the CBA ($H\text{-}2^k$) line used in the bulk of experiments. Therefore, splenocytes of mice with $H\text{-}2^k$ haplotype, including F_1 with other lines, lyse infected L929 ($H\text{-}2^k$) cells but neither virus free targets nor cells infected with an unrelated virus. The splenocytes of the immune mice were incapable of doing so. Furthermore, it was important to show that the immune splenocytes from other strains can kill infected cells with respective MHC type. Indeed, this was demonstrated using peritoneal macrophages from various strains as targets since these cells can be easily infected and labeled with ^{51}Cr. The immune T lymphocytes of $H\text{-}2^b$ mice lysed only infected macrophages from $H\text{-}2^b$ mice and had no effect on macrophages from animals with different haplotypes.

By early 1970s the biological function of transplantation antigens was unknown. These antigens were determined mainly by P. Gorer, G. Snell and others who obtained a number of murine lines for studies of transplantation rejection [4, 5]. J. Dosset and J. van Rood have found the respective antigens on the surface of human lymphocytes and suggested the term human lymphocyte antigens (HLA) [6, 7]. Immunophenotypic screening of human population showed that predisposition to some diseases is associated with the types of transplantation antigens. B. Benacerraf, H. McDevitt and F. Lylli discovered that inbred strains of guinea pigs and mice differ in their response to certain antigens and tumor cells [8-11]. Since a broad array of murine strains was available, such differences were easily detectable in MHC and its subregions. Identification of MHC class II loci as the immune response (Ir) genes was an important step to understanding the physiological role of MHC. These genes encode the humoral immune response to individual synthetic polypeptides [9]. By the time of publication of the works of Zinkernagel and Doherty these problems were hotly discussed. It was hypothesized that MHC polymorphism allows for preventing the transmission of tumor cells [12] or to avoid mimicking of transplantation antigens by viruses, thereby keeping the species identity [13, 14]. Also, transplantation antigens were supposed to generate the diversity of immune receptors. This hypothesis of N. Jerne stated that stem cells possess the complete number of V-genes that code for immune receptors that recognize all histocompatibility molecules of the species. Proliferation in the primary lymphoid organs leads to the suppression of “prohibited” autoreactive clones and selection of mutants with altered V-genes. Another part of the repertoire with specificity to alien transplantation antigens is responsible for autoaggression. The most provisional thought was offered by H. Lowrence (1959) who suggested that the infectious agents interact with transplantation antigens and form the complexes of “self” and “alien”.

Dual specificity of T cells to MHC and virus discovered by R. Zinkernagel and P. Doherty was a pivotal finding that unveiled not only the role of MHC but also explained previous phenomena of T cell responses to LCMV and leukemia and ectromelia viruses. The clear-cut analysis in vitro, the parallel work by G. Shearer demonstrating preferential recognition of trinitrophenol (TNP) labeled syngeneic targets by T cells that were immune to TNP [17], reproducibility of data using ectromelia and cowpox vaccine viruses [18, 19], H-Y antigen [20] and minor histocompatibility antigens [21] - all these studies convinced that MHC restricted recognition

is not a casual event but rather general biological mechanism. The major role of MHC molecules was promulgated as the signaling to the immune system about the changes in self MHC [22].

The MHC restricted manner of CTL-target interaction was expanded to helper cells, suggesting that they could recognize the antigen induced changes of MHC class II on B lymphocytes and macrophages. Most importantly, it became clear why MHC molecules are polymorphic: this diversity minimizes an opportunity of an non-immunogenic modification of these molecules, so that immunological tolerance of the entire population becomes practically improbable. However, only by mid-80s the works of E. Unanue, H. Gray, A. Townsend and J. Maryanski demonstrated that transplantation antigens encoded by MHC are antigen-presenting molecules; these molecules are recognized as complexes with antigenic peptides [23-26]. The latter ones were identified by H.-G.Rammensee et al. who eluted from MHC of target cells [27] and by crystallographic studies of P. Bjorkman, J. Wilson and D. Whiley who demonstrated a groove for peptide binding on MHC molecules and then made an X ray reconstruction of TCR-MHC-peptide complex [28, 29].

2. Origin of MHC restriction: adaptive differentiation or consequence of priming?

MHC restriction is an experimental observation of T lymphocyte recognition of the antigen in association with particular MHC encoded allelic product but not with the product of the another allele. This definition is provided by R. Schwartz in chapter 15 of "Immunology" by William Paul (1984) [30]. This book came out 10 years after the discovery of MHC restriction and 12 years prior to the decision of the Nobel Committee, so the above statement echoes the intense discussions of the problem in those times. The hypotheses of the origins of MHC restriction were the most disputable. The author of the chapter attempted to present as many viewpoints as possible which made the text difficult to comprehend, so I recommend this reading only to the highly motivated researcher.

The defenders of so called adaptive differentiation or ontogenetic model of MHC restriction based their theory on T cell recognition of MHC molecules presented in the body [31]. ***According to this model, T lymphocytes are normally restricted only by syngeneic MHC molecules because only these molecules are present during T cell maturation.*** This hypothesis is supported by experiments with bone marrow chimeras after transplanting the bone marrow of F_1 hybrids to irradiated parent animals (recipients) [32-34]. Recipients responded predominantly to the antigens associated with the recipient's MHC molecules (e.g., minor histocompatibility antigens and cowpox vaccine virus). These results contradicted the model of primary immunization that postulated the ability of T cells to recognize an antigen in complex with MHC molecules of any haplotype [35, 36]. ***The hypothesis of primary immunization claims that MHC restriction is a consequence of selection of T cells that proliferate in response to an antigen, and this proliferation is a result of primary recognition of a particular MHC molecule-peptide combination.***

General approach used in the first group of experiments was as follows: recipients of parent murine strain P1 were lethally irradiated, them their immune system was restored with bone marrow transfer from semiallogeneic recipients F_1 after thorough removal of T cells. The resulting chimerae contained hematopoietic cells from the donor (F_1) whereas other cells including thymus are of P1 origin. In these animals the repertoire of mature T cells was more narrow than in donors. The chimeras recognized predominantly the antigens in context with MHC molecules of the recipient. This fact was observed first by M. Bevan who studied the response to minor histocompatibility antigens [32] and then by R. Zinkernagel in response to cowpox vaccine virus of C57BL/6 chimerae with bone marrow from F_1 (bm1 x B6) [33]. However, R. Blanden and M. Andrew found that in these chimeras restriction to the recipient's MHC is not absolute. Among 53 chimeras that responded to ectromelia virus with CTL activation only 10 showed restriction to MHC of P_1 recipient whereas other chimeras lysed infected targets with P_1 and P_2 haplotypes [37]. The key experiments that demonstrated the role of the thymus in the development of MHC

restriction have been performed by R. Zinkernagel. The P1 recipients underwent thymectomy, then these mice were irradiated lethally followed by transplantation of T cell depleted bone marrow from F_1 mice. Then the lobes of irradiated thymus from F_1 hybrids, P_1 or P_2 were transplanted to these animals. The specificity of restriction of CTL induced by cowpox virus depended on MHC phenotype of radioresistant cells of transplanted thymus [38, 39].

It has also been demonstrated that the specificity of restriction of T cell repertoire in semiallogeneic chimerae can be largely dependent on the time course of the appearance of bone marrow derived antigen presenting cells (APC) in the recipient's thymus. In these chimeras APC of the donor type (F_1) can be found in the recipient's (P_1) thymus two months after bone marrow transplantation. If by this time to deplete the peripheral T cells restricted in MHC P_1, new T cells that migrated from the thymus are restricted in MHC of both parents [40]. Eventually it was confirmed that positive selection in the thymus is not critically dependent on thymic epithelium and can be regulated by other cell types [41, 42]. Moreover, positive selection can proceed without MHC molecules and does not require their signaling via a co-receptor after interacting with antibodies to CD3 or clonotype. The only requirement for positive selection is low affinity binding of TCR that causes no aggregation of this receptor [43].

The efforts to reproduce the results with semiallogeneic chimerae on fully allogeneic ones were unsuccessful. The allogeneic chimerae develop the repertoire restricted either to the donor or to the recipient MHC molecules [44]. In another set of experiments the thymus from P_1 was transplanted to F_1 after thymectomy, then mice were irradiated and their bone marrow was restored with that from P_2 after T cell depletion. The animals responded to an alloantigen, indicating that the functional T cell repertoire was formed. After in vivo immunization and subsequent restimulation with inactivated Sendai virus, CTL restricted to P_2 MHC were registered in all cases whereas CTL from P_1 (the donor of the thymocytes) were seldom [45].

Also, the attempts to show the influence of transplanted allogeneic thymus on MHC restriction of T cell repertoire in *nude* mice. Transplantation of allogeneic thymus to *nude* mice led to a repertoire restricted to MHC molecules of the recipient [46].

Apparent contradictions in experimental data were discussed in detail by A. Singer and P. Matzinger. The critical questions were: 1) do the results on chimerae reflect real positive selection of T cell repertoire by MHC molecules of thymic epithelium, and is this selection independent of the immunizing antigen? 2) what are the 'suppressor mechanisms' caused by allogeneic combination of bone marrow cells, transplanted thymocytes and irradiated or thymectomized recipients? 3) is specificity determined solely by immunizing antigen presented by bone marrow APC [47,48]. Despite the controversial results obtained on radiation chimerae, the immunological community accepted the hypothesis of “adaptive differentiation in the thymus”, and this hypothesis is currently presented in all text-books. The conflicting data on *nude* mice were explained by the presence of rudiments of the thymus as well as “residual” differentiation and selection of T cells in these animals. It is noteworthy that these suggestions had not been supported by any morphological data.

3. MHC restirction in *nude* mice: lessons from animals with knocked out recombinazes and aggregation chimerae.

The problem of MHC restriction in *nude* mice was thoroughly investigated by R. Zinkernagel when RAG knockouted and TCR transgenic mice became available. Because of high fidelity of these experiments they must be discussed in detail. In the first series *nude* F_1 (H-2^b x H-2^d) were irradiated sublethally (4.5 Gy) followed by transplantation of embryonic thymus H-2^b RAG-1 or H-2^d SCID or H-2^d RAG-$1^{0/0}$. After 12-16 weeks these mice were immunized wth LCMV. At day 8 post immunization the restriction of CTL was tested. The response was restricted to thymic MHC molecules and led to the complete disappearance of the virus from the spleen and liver. The results confirmed earlier data and showed that “biase” of MHC restriction due to transplantation of thymocytes could not be associated with specific “suppressor mechanisms” because only thymi of

immunodeficient mice were used for reconstitution. In the second series mice of H-2^b haplotype were rescued with transplanted fully allogeneic thymus H-2^k RAG-$1^{0/0}$. As before, CTL activity was restricted to MHC molecules of the recipient but not those of the thymus; the virus was also eliminated. Furthermore, the authors addressed the situation that in radiation chimerae a certain amount of hematopoietic cells could survive and thereby influence positive selection. Together with transplantation of allogeneic H-2^k RAG-$1^{0/0}$ thymus, bone marrow cells from the same immunodeficient donors were inoculated. Surprisingly, the specificity of recipient's T cells (note that recipient were chimerae whose hematopoietic cells carried half recipient and half donor haplotypes) was restricted to MHC molecules of the donor and recipient and, in some cases, only to MHC molecules of the donor. The authors concluded that ***there is an alternative (if not major) way of selection and maturation of T cells that depends on bone marrow derived cells*** [49]. In the same work it has been shown that the expression of MHC molecules in the transplanted thymus used for reconstitution of H-2^b mice is not necessary for restoration of T cell repertoire. Transplantation of the embryonic thymus from double class I and II knockouts or beta-2 microglobulin knockouts led to normal CTL response to LCMV restricted to H-2^b haplotype. Even more surprisingly, the complete restoration of the response was achieved after transplantation of the thymus from Lewis rats, the xenogenic donors. Therefore, ***the thymus acts mainly as an organ for differentiation, rearrangement and expression of the TCR genes***. This statement was tested in experiments with transgenic TCR318 specific to LCMV-GP 33-41 in the context of D^b. Positive selection of this transgenic receptor occurs only in H-2^b but not in H-2^q mice. So, positive selection and T cell expansion should take place in *nude* mice that express D^b on bone marrow cells. Indeed, the expansion of $CD8^+$ T cells (>90% positive for the transgene) was observed in *nude* mice with H-$2^{b/q}$ haplotype but not H-$2^{q/q}$. Accordingly, after infection with LCMV the CTL $CD8^+$ response was observed in H-$2^{b/q}$ but not in H-$2^{q/q}$ mice. So, ***specificity of T cells reflects the expansion of the repertoire and viability of T cells on the periphery, as well as the induction of effectors by MHC molecules on the bone marrow derived cells***.

In their subsequent brilliant work Marianne Martinic and Rolf Zinkernagel (2003) used an original approach to make aggregation allogeneic chimerae of 8 cell embryos, one of which contained *nude* homozygote and another one RAG-$1^{0/0}$ or SCID homozygote. The chimerae SCID H-2^d + *nude* H-2^b and RAG-$1^{0/0}$ H-2^b + *nude* H-2^d were obtained; the thymic epithelium of these mice has the haplotype of MHC of SCID or RAG-$1^{0/0}$ embryo whereas T and B lymphocytes originate from the *nude* embryo. These mice develop mixed chimerism in various tissues, as determined by the expression of glucoso-6-phosphate isomerase isoforms. Among leukocytes $CD4^+$, $CD8^+$ and $B220^+$ cells had *nude* haplotype whereas two populations of $CD11b^+$ macrophages expressed the haplotypes of each parent. The thymi of these chimerae were subjected to immunohistochemical analysis that showed normal cellular content and well differentiated epithelium with RAG-$1^{0/0}$ haplotype. To rule out thymic rudiments, a double staining for MHC molecules and cytokeratin was performed to demonstrate co-expression of class II MHC molecules of RAG-$1^{0/0}$ parent and cytokeratin on the same cells. In contrast, similar double staining with antibodies against MHC molecules of *nude* and cytokeratin revealed class II MHC with *nude* haplotype and no cytokeratin expression. This indicates the presence in the thymus of non-epithelial (i.e., hematopoietic) cells with MHC molecules from *nude* parent. None of the chimerae had thymic rudiments with mature epithelial cells carrying *nude* haplotype. Infection of the chimerae with LCMV and subsequent CTL response to H-2^b and H-2^d restricted peptides showed that ***T cell repertoire of aggregation chimerae is restricted to MHC molecules of both parental haplotypes***. The response was commensurating with that of wild type mice and led to the complete clearance of the spleen and other organs. To better characterize the repertoire of $CD8^+$ effectors, they were stained with MHC tetramers to show two distinct populations positive either for LCMV-GP33 (H-$2D^b$) or for LCMV-NP118 (H-$2L^d$). Therefore, double restriction of the repertoire is associated with real changes in specificity of restriction and not with cross-reactions. Also interesting is the development of efficient humoral IgG response in chimerae infected with vesicular stomatitis virus (VSV) and LCMV, and this response is comparable with that in wild type

mice. Animals responded with the production of neutralizing anti-VSV and anti-LCMV nucleoprotein which is strictly dependent on the cognate MHC restricted $CD4^+$ helper function. As mentioned above, B cells of the chimerae express MHC of the *nude* parent. This means that ***in aggregation chimerae $CD4^+$ helpers are restricted by non-thymic MHC molecules***. The apparent contradictions with the results on semiallogeneic radiation chimerae were explained by incomplete elimination of TCR interacting host cells after irradiation; thus, host T cells survive regardless of their location in the thymus or on periphery. Since proliferation in the thymus is very active, these host T cells can get an advantage over transplanted donor cells that must first migrate to the thymus. The aggregation chimerae are preferable because populations developing on thymic and non-thymic MHC are in equal start conditions [50]. In other words, early data on radiation chimerae are artifacts, and the ontogenetic model of MHC restriction is therefore doubtful. Most likely, this model holds true only to dependence of T cell survival on the periphery on MHC molecules. ***Altogether, MHC restriction of T cell repertoire is regulated not as much as by MHC haplotype of thymic epithelium but by the haplotype of bone marrow cells, (probably, professional APC that present the antigens to T lymphocytes. – author's remark***)

4. Adaptive differentiation and positive selection: novel approaches.

Among basic findings for ontogenetic model of MHC restriction were the results showing that some class II molecules can increase the frequency of mature peripheral T cells expressing individual Vβ regions [54, 55]. Furthermore, blocking individual allelic MHC products in F_1 hybrids with allele specific antibodies inhibited helper and CTL responses restricted to the blocked allele [56, 57]. Importantly, the absence of MHC class I prevented $CD8^+$ formation whereas the absence of class II prevented the development of $CD4^+$ [58-61].

Generation of animals with transgenic TCR could prove the above hypothesis. These mice develop high number of T cells with certain antigenic and restriction specificity, and these characteristics can easily be controlled by various MHC environment. The initial works on positive selection of transgenic H-Y TCR (specific for sex antigen peptide in the context of $H\text{-}2D^b$) [62,63], 2C (specific for $H\text{-}2L^d$) [64] and AND (specific for pigeon's cytochrome c fragment in the context of $I\text{-}E^k$) [65, 66] allowed to conclude that the mature T cell with the transgenic receptor can develop in the presence of the restricting MHC allele and the absence of specific peptide. This link is the most clear in mice with transgenic TCR specific to H-Y antigen. The plots showing positive selection of $CD8^+$ with this TCR in the thymus of $RAG^{0/0}$ $H\text{-}2^{b/d}$ and no selection in the $RAG^{0/0}$ thymus $H\text{-}2^{d/d}$ can be found in many text-books of immunology. These data directly demonstrate the link between positive selection and MHC restriction of the repertoire.

Eventually it became apparent that this viewpoint is somewhat idealized. Positive selection of $CD8^+$ cells with transgenic H-Y TCR in $H\text{-}2^b$ mice appeared to be associated with the loss of $CD4^+$ cells with the same receptor. A reason for this fact is the ability of the receptor to react with A^b molecule presented in mice of the same haplotype [67]. Studies of selection of 2C-TCR in wild type B6 mice, K^b mutants and animals with the initially expressed L^d alloantigen revealed at least five phenotypic patterns of T cell selection. These included 1) positive selection (K^b and K^{bm7}); 2) weak positive selection (K^{bm8}); 3) no positive selection (K^{bm1} and K^{bm10}); 4) negative selection of $CD8^{hi}$ (K^{bm3} and K^{bm11}); and 5) negative selection of all $CD8^+$ cells ($H\text{-}2L^d$). These results show the direct interaction of 2C-TCR with various allelic forms of MHC molecules in the course of positive and negative selection [68]. Also, 2C TCR can recognize, besides the immunizing complex of L^d with peptide p2Ca (LSPFPFDL), the K^{bm3} with peptide dEV8 (EQYKFYSV) and positively selecting K^b molecule associated with peptide SIYR-8 (SIYRYYGL) [69-71]. Recognition of peptide antigens by this receptor is specific in the context of allogeneic L^d molecule, whereas in the context of positively selecting $H\text{-}2K^b$ molecule all three peptides showed degenerative recognition [72]. Moreover, positive selection of TCR can be observed in bm3 $TAP^{0/0}$ mice, i.e., on "empty" heavy chains of K^{bm3} [73]. Also, it was established that positive selection of T cell receptor AND

can proceed on different MHC alleles [74]. Thus, these findings contradict the expectations of "adaptive differentiation" adepts.

As mentioned by Diane Mathis and Christophe Benoist in chapter 11 of William Paul's "Fundamental Immunology", animal experiments with transgenic TCR seldom give black-and-white results. Indeed, some TCRs can be positively selected by other MHC alleles than the restricting one, and positive selection of certain receptor is inefficient even by the selecting allele [75]. Furthermore, narrowing of the repertoire down to CD4 or CD8 cells is far from being absolute [76, 77]. In recent work Nadezda Logunova and Alexander Chervonsky gave an interesting exemple of such receptors by showing TCR MM14.4 obtained in response of transgenic mice with limited repertoire of presented peptides to the syngeneic MHC class II I-A^b molecule. Transfer of the transgene to wild type C57BL/6 mice led to deletion of T cells with this receptor via negative selection. Although the receptor was initially cloned from T cell hybridoma $CD4^+$, predominantly $CD8^+$ cells were positively selected in mice expressing the individual complex A^b with Eα chain AA52-68. In mice totally lacking MHC class II $CD4^+$ cells were absent; in mice without MHC class I molecules $CD8^+$ cells were absent. Positive selection of $CD4^+$ T cells with TCR MM14.4 was observed on three alleles of MHC class II molecules: in BALB/c (H-2^d), A_β^{bm12} mutants and DM knockouts with A^b complexed with CLIP peptide of invariant chain (li). These facts suggest degenerative recognition of MHC molecules during positive selection. Moreover, MM14.4 expressing mice frequently developed $CD8^+$ T cell mediated autoimmune dermatitis. In vitro MM14.4 $CD8^+$ cells were alloreactive with allogeneic molecule H-$2K^k$ [78].

Recent works on "specificity" of positive selection were aimed at generating transgenic animals expressing individual complexes MHC/peptide. The net result of these works shows that limitation of the repertoire of presenting peptides lowers the efficiency of positive selection and even presentation of endogenous superantigens [79]. Nevertheless, selection of diverse repertoire of T lymphocytes occurs in these mice, and T cells are capable of reacting on different allelic forms of MHC molecules [80-83].

From the above cited works one can conclude that ***positive selection of the repertoire is a result of degenerative recognition of endogenous MHC/peptide complexes. Although the efficacy can depend on variety of peptides associated with 'self' MHC molecules, positive selection cannot determine the restriction specificity of the forming T cell repertoire***.

5. Allorestricted recognition as evidence in support of "priming" hypothesis.

The most important conclusion made by the adepts of "adaptive differentiation" hypothesis after the experiments with semiallogeneic chimerae, was the following: MHC restriction is a phenomenon adopted by T lymphocytes in the course of antigen independent differentiation in the thymus. This ontogenetic event "narrows" T cell repertoire to the extent that only clones specific to the antigen associated with self MHC molecules proliferate in response to this antigen. Therefore, this model presumes that the non-immune population of T cells does not recognize the antigen associated with allogeneic MHC molecules. This statement is in controversy to "priming" hypothesis that surmises T cell clones that recognize the antigen associated with allogeneic MHC molecules. So the crucial question arised in the collision of the two hypotheses is whether there is allorestricted recognition, i.e., can T lymphocytes generated in a particluar MHC environment recognize the antigens presented on allogeneic APC?

This question appeared to be difficult to answer. Allogeneic effects were a major obstacle for experimentation because the number of clones that recognize an individual antigen is some two orders of magnitude smaller compared with clones reacting to allogeneic MHC molecules. Initially this problem was addressed by method of filtration or "acute depletion" of alloreactive cells. T cells were injected into the irradiated allogeneic recipient. Alloreactive T cells repopulated the lymphoid organs and temporarily (for 2 days) lost the ability to circulate whereas other T cells could migrate and could be isolated from ductus thoracicus [84]. Studying the specificity of CTL to cowpox and influenza viruses, P. Doherty and J. Bennink "filtered" the cells from H-2^k through irradiated F_1 (H-

$2^{k/b}$). The T cell population obtained after negative selection in irradiated F_1 recipients was stimulated with the virus. The resulting CTL were specific only to syngeneic (H-2^k) but not to allogeneic (H-2^b) targets infected with the virus whereas in normal F_1 mice the CTLs specific to infected targets of both haplotypes were found [85]. One can suggest that these data showed that MHC restriction is a basic feature gained during ontogenesis. However, further work of these investigators demonstrated the recognition of the virus in the context of MHC molecules of certain haplotypes, and that this effect is not a consequence of cross reactivity [86]. The modifications of in vitro "acute depletion" method were based on the absorption of alloreactive T lymphocytes on monolayers of allogeneic cells. In the work of Hubertus Stockinger and Hermann Wagner this approach was coupled with the method of limiting dilutions for quantitative analysis of MHC restriction of the repertoire at the level of individual clones. Studying the restriction of the response of allogeneic chimerae to trinitrophenol modified targets or Sendai virus infected ones showed that, depending on the protocol of immunization, the response could be restricted to H-2 antigens of either the donor of stem cells or recipient's thymus. It appeared that T cell populations of normal mice in which alloreactive cells are depleted, contain CTL precursors capable of specifically reacting with the virus or trinitrophenol derivatives in the context of allogeneic MHC absent during intrathymic differentiation. Using the method of limiting dilutions, the authors determined the frequency of auto- and allorestricted CTL in this depleted population. The frequency of precursors with syngeneic restriction was approximately 6 times higher than that of precursors with allogeneic restriction. This difference fluctuated from 2 to 10-fold depending on the combination of inbred strains [35, 36]. Similar results were observed by the same authors for precursors of thymus CTL devoid of alloreactive cells and recognizing trinitrophenol derivatives in the context of syngeneic and allogeneic MHC molecules [87]. Harald von Boemer et al. criticized these studies because has got no convincing evidence for recognition of minor histocompatibility antigens in the context of allogeneic MHC molecules. In F_1(CBA x B6) chimerae reconstituted with B6 bone marrow, the ratio of clones recognizing BALB/b (H-2^b) and BALB/k (H-2^k) targets was 3:1 while in normal B6 mice it was 17:1. Also, in normal mice there was cross reactivity of the clones that recognized BALB/k with the clones that recognized BALB/b. Such cross reactivity was found in 25% clones from normal mice and only in 1% of clones from chimerae [34]. Wagner was right to suggest that the preference of T lymphocyte repertoire for antigen recognition in the context of "self" MHC can be a subsequence of an experimental procedure and "breaking" normal repertoire in the course of chimerism formation or depletion of alloreactive cells. This is why in next two works by Jorg Reiman and Hermann Wagner determined the frequencies of allorestricted clones in the repertoire of normal allogeneic animals. Using six combinations of allogeneic strains it was shown that stimulation of CTL precursors with TNF-modified allogeneic cells caused unusually high frequencies of clones that react with these CTL (1/30-1/300); the CTL reacting clones did not react with non-modified allogeneic targets and were therefore allorestricted [88]. The responses of combinations of B6 and bm1 stimulators and responders to herpes simplex virus and trinitrophenol derivatives showed that about 30% of reacting clones recognize their targets in a allorestricted manner, i.e., they do not react with non-infected or non-modified targets [89]. D. Kabelitz and J. Reiman demonstrated the existence of allorestricted T cells in humans that respond to parotitis virus [90]. Other groups also reported on cells that specifically recognize the antigenic peptide in the context of allogeneic MHC molecules [70, 91, 92].

The restriction of responses of allorestricted T cells to MHC molecules absent in the thymus was used for obtaining high avidity clones capable of recognizing tumor associated antigens in patients that express the respective MHC molecule. Indeed, normally negative selection ablates high avidity T cell clones that can react with self antigens of an organism P1 in the context of self MHC molecules (H-2^x-P1). But the clones specific to H-2^x-P1 can be presented in an allogeneic organism P2 because negative selection in this organism deletes the clones specific to tumor associated antigens in the context of "another self" (i.e., allogeneic to P1) MHC (H-2^y-P2). Therefore, allorestricted recognition can supposedly provide the basis for obtaining clones of P2 specific to the combination of MHC molecule with H-2^x-P1 peptide of allogeneic recipient for

adoptive immunotherapy. One example of successful use of allorestricted recognition of tumor associated antigens is the work of Elena Sadovnikova and Hans Stauss who obtained allorestricted CTL clones of H-2^d mice. These clones were specific to the complex of H-$2K^b$ plus mdm-2 derived peptide; it is noteworthy that mdm-2 is frequently overexpressed in tumor cells. In culture these clones selectively reacted with tumor versus normal cells and killed melanoma and lymphoma cells but not normal H-$2K^b$ expressing dendritic cells. In vivo the allorestricted clones caused retardation of growth of melanoma and lymphoma cells in the syngeneic (H-2^b) recipients [93, 94]. The same authors attempted to obtain allorestricted clones specific to a cyclin D1 peptide in the context of human HLA-A2. The clones lysed cyclin D1 overexpressing breast carcinoma cells but not Epstein-Barr transformed lymphoblastoid cells [95]. ***Allorestricted recognition became an efficient means of breaking tolerance to tumor associated antigens and to get responses to leukemia associated markers such as WT1, CD68 and CD45*** [96-98].

During last years a significant impact into the theory of allorestriction has been made by Rammensee and colleagues. Previously this researcher had identified MHC binding motifs in peptides that interact with allele specific forms of MHC molecules. The approach of Reinhard Obst et al. was to stimulate the repertoire of T cells H-2^d with a mixture of synthetic peptides from combinatorial peptide libraries. The peptides had an MHC binding motif for interaction with H-$2K^b$ molecule. For presenting the synthetic peptides on APC, the cells lacking TAPs were used. Incubation of these cells with the peptides whose structure is optimal for binding with MHC results in successful formation of the complex MHC molecule/β_2-microglobulin/peptide and subsequent transport onto the plasma membrane. Allorestricted as well as autorestricted CTL lines obtained in response to such cells widely varied in their peptide specificity and avidity. The authors concluded that positive selection in the context of certain MHC molecule is not required for generating high avidity TCR restricted to the same molecule but increases the frequency of these CTL. Similarly, the authors analyzed the precursors of allorestricted CTL in peripheral blood of HLA-A2 and HLA-A3 negative donors. The response was induced by TAP negative targets that express these HLA molecules after incubation with combinatorial peptide libraries containing proper MHC binding motifs. The CTL specific to these peptide libraries in the context of allogeneic MHC molecules comprised a major part of the repertoire, namely, ~50% of all alloreactive clones. However, the frequency of allorestricted CTL was also twice as lower than the frequency of CTL restricted to self MHC molecules [99, 100]. In the subsequent work the authors studied the link between the expression of self MHC molecules and alloreactive and allorestricted repertoire; the methodological approach was as above plus testing allorestricted responses to known viral and self peptides. The closer were the structures of allogeneic MHC molecule and T cell MHC molecule, the higher is the ratio of allorestricted CTL that recognize antigen peptides to CTL recognizing the allogeneic molecule independently of the peptide. This ratio was ~1/5 for totally allogeneic combination H-2^d-anti-H-2^b and 1/3 in the response H-2^{bm1}-anti-H-2^b. As expected, the highest ratio of peptide specific clones - 3/1 - was found in the response to H-2^b stimulators of mutant bm13 and bm14 with the mutations in the antigen binding groove of H-$2D^b$ molecule. This link could well be associated with the effect on the alloreactive repertoire of positive selection in the thymus and T cell survival on the periphery. However, this link does not prohibit the recognition of peptides in the allogeneic context [101]. Recently, using MHC tetramer technology, the allorestricted T lymphocytes that specifically recognize antigenic peptides were directly visualized and isolated [102].

Thus, based on above mentioned facts, T cells recognize antigens in the context of MHC molecules that are absent during thymocyte differentiation. Apparently, the phenomenon of allorestricted recognition is confirmed by a plethora of data from many laboratories. This phenomenon extends far beyond single clone specificity or the opinion of individual scientists. Moreover, in its broadness, diversity and specificity of recognition, the allorestricted repertoire of T cells is comparable with the repertoire restricted to self MHC molecules. The existence of T cells capable of recognizing antigens in the context of allogeneic MHC molecules is by itself a solid argument against the hypothesis of ontogenetic origin of MHC restriction. Of note, positive

selection in the thymus and T cell survival on the periphery play minimal role in the formation of T lymphocyte specificity, much less important than expected. This makes the hypothesis of adaptive differentiation inappropriate for explaining the experimental phenomenon discovered by Zinkernagel and Doherty.

6. The molecular basis of MHC restriction: MHC binding motifs.

Can the hypothesis of primary priming explain difficulties and controversies of data that adaptive differentiation cannot? The dependence of repertoire restriction on replacement of host APC with donor cells (found in radiation chimerae [40]) and simultaneous transplantation of the thymus and bone marrow from RAG knockouts to *nude* mice [49] lead to quite logical assumption that MHC restriction is controlled at the level of antigen presentation to T cells. Indeed, measurements of specific immune functions of effector cells always required priming of naive T cells with the antigen. The "non-thymic cells originated from the bone marrow" that participate in the immune responses are professional APC: dendritic cells, B cells and macrophages. Different lifespan of these cells after lethal irradiation and different role in the immune response might well be a source of experimental artifacts and misinterpretations. On the other hand, it is evident that easily detected primary responses (such as allogeneic response or reaction to bacterial superantigens) are MHC-unrestricted.

The mechanism of how APC determine the restriction of effector T cells was uncovered by the Hans-Georg Rammensee group. As the author mentioned in his review on MHC binding motifs in antigens, immunology owes two students, Olaf Roetzke and Kirsten Folk, for their discovery. Both guys were interested in the structure of peptides that interact with class I MHC molecules. To isolate these molecules, proteins of cell membranes were adsorbed on an immunoaffine column followed by acidic elution and dissociation of MHC/β_2-microglobulin/peptide complexes. The Edman sequencing of isolated peptides showed invariant amino acid residues near from the C and N ends. Most importantly, peptides bound by different allelic forms of MHC class I molecules had similar length but different allele-specific motifs [27, 103, 104]. The authors realized that they possess a powerful method that allows to identify the allele-specific sequences among a huge variety of peptides which could derive from one antigenic protein [105].

The fact that these motifs have been formed by "anchoring" amino acid residues necessary for high affinity binding of the peptide with respective MHC molecule also indicated that APC expressing different MHC haplotypes will present various peptides of the same antigen [106]. For example, nucleoprotein of influenza virus contains the epitope that binds with H-2K^d in positions AA147-155 (T**Y**QRTRAL**V**) and with H-2D^b in positions AA366-374 (MTEM**N**ENS**A**). For human MHC molecules the epitope for binding with HLA-A2 is within AA85-94 (K**L**GEFYNQ**M**), with HLA-A3 within AA265-273 (I**L**RGSVAH**K**), with HLA-B8 within AA380-388 (IAWY**R**SR**L**E), with HLA-B27 in AA383-391 (S**R**YWAIRT**R**) (underlined are anchoring, i.e., motif forming, residues). The highest affinity of binding of influenza virus nucleoprotein derived peptides with H-2D^b is achieved if the peptide has the canonical motif [107]. To address the question of the expression of certain MHC molecule for the efficacy of presentation of peptide epitopes, differences in the amounts of correctly processed K^b restricted epitope of the minor histocompatibility antigen H-4b in H-2K^b positive and -negative cells were estimated. The difference was 3000-fold, indicating an instructive role of MHC molecules in peptide processing machinery [108]. The mechanisms of peptide/MHC molecule association allowed to predict the structure of T cell peptide epitopes including tumor antigens [109-111]. Furthermore, these studies set rational molecular basis for an association between autoimmune diseases and certain MHC haplotypes [112-114]. Finally, the ability of individual allelic products of MHC molecules to bind particular peptides of the pathogen directly links MHC with genetically determined immune response to pathogens.

The experience gained in the studies of MHC binding motifs in MHC associated peptides is currently used for constructing combinatorial peptide libraries. These tools make possible the

positional screening for high affinity ligands and cross reacting ones for T cell receptors and making of MHC tetramers which can be identified for direct visualization of antigen specific T lymphocytes [115, 116]. There is a number of on-line services making possible the search for T cell epitopes among different protein sequences. One of them RANKPEP service is powerful mean for search of potential T cell epitopes presented by allelic forms of mouse and human MHC classes I and II molecules taking into account cutting of proteins by proteasoma [117].

Importantly, the H.-G. Rammensee group has discovered the molecular mechanism of MHC restriction. In initial experiments the immunization of CBA (H-2^k) mice with LCMV have induced CTLs that recognized viral peptides with MHC H-2^k binding motifs. Clearly, these specific CTL lysed infected L929 cells or macrophages (H-2^k) that presented the same viral peptides. Certainly, these CTLs did not kill infected macrophages of H-2^d haplotype that present totally different peptides of the same virus. The same holds true for the reverse combination. Immunization of H-2^d mice with LCMV induces CTL specific to H-2^d binding LCMV peptides. These CTLs would lyse H-2^d targets that presented the same viral peptides but not H-2^k targets that presented different peptides, i.e., those with H-2^k binding motifs. In contrast, immunization of F_1 (H-$2^{k/d}$) mice generates two independent CTP populations that lyse H-2^k as well as H-2^d targets correspondingly. This fact has been formally demonstrated in the analysis of immunogenicity of three LCMV epitopes restricted by H-2D^b molecule, namely, GP33-41 (KAVY**N**FATC), GP276-286 (SGVE**N**PGGYCL) and NP396-404 (FQPQ**N**GQF**I**). The efficacy of presentation of these peptides correlated with the intensity of antiviral CTL response. The NP396-404 peptide containing two anchoring residues for H-2D^b binding) showed the highest protective effect regardless of its relatively low amount on APC [118].

The results analyzed in this section indicate that MHC restriction can be explained by "primary immunization" hypothesis which presumes the specificity of effector cells activated during primary response. Difference of allele specific motifs for binding of peptides with MHC molecules comprises the molecular basis of this phenomenon.

7. Origins of allogeneic response: direct and indirect recognition.

Controversial character and internal conflicts in interpretations of modern immunology are especially evident in the field devoted to studies of allogeneic responses, i.e., the responses to transplantation antigens of genetically alien organisms within the same species. All known transplantation antigens fall into two categories: major histocompatibility antigens (i.e., classical H-2 encoded MHC molecules) and minor antigens, i.e., other polymorphic transplantation antigens .

The allogeneic MHC molecules are the most important for the rejection of the transplant. In early works it has been shown that the transplant with foreign MHC antigens was rejected by days 8-10 whereas the transplants with alien minor antigens remain viable for at least three weeks. The MHC alloantigens induce very strong T cell responses in culture, namely, primary responses although the responses to conventional antigens, such as ovalbumin, require the preliminary immunization. Furthermore, the alloreactive precursors are more frequent than cells specific to the antigens presented with self MHC molecules. The frequency of alloreactive T cells can be as high as 2-5% of total T lymphocyte population whereas T cells that react to soluble or viral antigens are normally 1:10,000 [119].

Two models were suggested to explain T cell recognition of the alloantigen. ***Direct allogeneic recognition*** presumes the interaction of T cell receptor with allogeneic MHC molecule bound to the peptide from allogeneic APC. It is widely believed that after grafting this response is mediated by migration of donor APC to the lymphoid tissue of the recipient. This model explains the fact that allogeneic response is not restricted by MHC. Also, the model is in concert with dominant genetic control of inducibility of the response to the transplant by the histocompatibility alleles. ***Indirect allogeneic recognition*** implies that the allogeneic peptide is recognized by T cells being bound to an MHC molecule of the recipient. This mechanism functions in the responses to

minor histocompatibility antigens and is MHC restricted. As in other MHC restricted responses, the inheritance here is co-dominant. This mode of recognition is a result of presentation of allogeneic peptides derived from the graft by dendritic cells of the recipient. After engulfment by dendritic cell proteins of the graft can be processed in endocytic compartment for subsequent presentation in the context of recipient's MHC class II or transferred into endoplasmic reticulum to be associated with MHC class I molecules for further cross-priming of the recipient's T lymphocytes [120-122].

Apparently, the hypothesis of adaptive differentiation is in agreement only with the second model whereas primary priming is concert with both models. According to adaptive differentiation, allogeneic recognition must be a consequence of recognition of allogeneic peptides in the context of self MHC molecules of the responder. Direct interaction with allogeneic MHC molecules can occur only as a random cross reaction of T cell receptors "instructed" to react with self MHC molecules.

Nevertheless, convincing evidence has been provided in support of both mechanisms of the response to the transplant. The detailed study of direct and indirect responses to alloantigens was performed by Gilles Benichou using ELISPOT, the method of detection of cytokine production by single cells. Indirect responses comprised 1-10% of total responses to fully allogeneic skin transplant of B10.A ($K^kI^kD^dL^d$) to BALB/c recipients ($K^dI^dD^dL^d$). Direct responses remained predominant (98%) in secondary response 6 weeks after the rejection of the transplant [123]. In the study of direct and indirect responses of $CD8^+$ IFNγ producing cells, the frequencies of T cells that directly recognize the alloantigen were 30-60-fold higher [124]. The mode of recognition depends on the type of the transplant, antigenic differences and availability of costimulatory APC ligands [125, 126]. However, the efficacy of indirect allogeneic recognition in the cited articles could be overestimated (see below).

Most likely, unambiguous acception of adaptive differentiation was the reason for numerous attempts to prove that indirect recognition is a key mechanism of response to the transplant even if the donor and the recipient differ solely in MHC whereas their minor histocompatibility antigens are identical. In general the adepts of this viewpoint admitted the predominance of direct responses. Indeed, murine and human MHC class II molecules frequently present the processed peptides of class II molecules itself [127-129]. On top of that, some MHC molecule derived peptides were identified as the participants of indirect allogeneic recognition [130-132]. To demonstrate indirect recognition, the authors lysed donor APC with hypotonic shock or ultrasound and freeze-thawing and added the lysates to MLR, in the setting the direct presentation of the antigen is impossible. The lysed APC of MHC incompatible donors induced T cell proliferation in mixed lymphocyte culture. However, this effect was clearly detectable only after preliminary immunization of recipients and was not confirmed by other arguments in favor of indirect mechanism, i.e., inhibition with antibodies against the presenting MHC allele, use of APC from MHC deficient mice and APC from recombinant mice that express other presenting alleles. Moreover, no phenotyping of proliferating cells was performed due to knowing for sure that in this MLR the a priori proliferating cells are $CD4^+$ [122].

To study the responses to allogeneic class I MHC molecules we used similar system in which C57BL/10 (H-2^b) mice were immunized with P815 mastocytoma (H-2^d) cells. Restimulation in in vitro MLR was performed two months later using stimulator splenocytes of C57BL/10 (H-2^b), B10.D2 or BALB/c (H-2^d) and C3H (H-2^k) mice; splenocytes were heat shocked. The primary proliferative response to dead allogeneic APC was absent, according to the above works. Dead allogeneic APC triggered T cell propliferation of preimmunized recipients. But in response to the immunizing antigen only $CD8^+$ cells of immune animals proliferated. Similar result was obtained in the system with B10.D2 (R101) ($K^dI^dD^b$) mice immunized with EL4 (H-2^b) thymoma cells followed by restimulation in in vitro MLR with heated splenocytes from B10.D2 (R101), C57BL/6 (H-2^b) and C3H (H-2^k) mice. We showed that the ability to proliferate in response to dead allogeneic APC was not a consequence of indirect recognition of the alloantigen but rather a specific feature of $CD8^+$ memory cells primed by the antigen and trans-costimulated. The tumor cells used in this study are not professional APC, so one can expect the predominance of the

indirect recognition of the alloantigen by $CD4^+$ cells. However, only $CD8^+$ cells proliferated in the tested response. Moreover, these cells efficiently inhibited the responses of naive $CD8^+$ cells and their involvement into memory cell pool. Primed $CD8^+$ cells recognized the antigen directly because the proliferation of R101 mouse memory cells was blocked by antibody to $H\text{-}2K^b$ and because the response was absent if stimulation in MLR was performed with cells from TAP and β_2-microglobulin knockouts on C57BL/6 ($H\text{-}2^b$) background [133, 134]. These results were supported by the analysis of memory cell responses to mutant MHC molecules. Recognition of $H\text{-}2K^b$ molecule by memory cells was impaired by the mutation in the peptide binding region (residue 77 of bm3 mutant) and in TCR binding region (positions 173 and 174 of bm4 mutant) but not by the mutation in class II MHC molecule (positions 67, 70 and 71 of $A_\beta{}^b$-chain) [135]. Thus, ***even if indirect recognition of allogeneic class I MHC molecules is favored (immunization with "non-professional" tumor APC), $CD8^+$ T cells directly interacting with foreign MHC class I molecule are a major component of the response.***

8. Origin of direct allogeneic recognition: "two signals - one APC" or "trans-costimulation"?

The above works are far from being the only evidence for direct allogeneic recognition. Perhaps Boris Brondz was the first to separate T cells on the basis of specific recognition of MHC products. To do this, he used the monolayers of allogeneic macrophages [136]. A bit less than 20 years ago evidence in support of direct recognition of alloantigens was obtained as a response of primed CTL to artificial membranes, that is, the lipid, H-2 antigen and alkylated particles of SiO_2 in deoxycholate. These "artificial cells" induced secondary, but not primary, CTL differentiation, and this response was specific and not less efficient than response to allogeneic cells. The processing of the antigen in APC and helper activation did not occur because the response was dependent on exogenous cytokines [137]. Later M. F. Mescher and K. P. Kein showed that cloned CTL can lose granules upon interaction with plastic adsorbed antigen [138]. Convincing proof of direct recognition of alloantigens on tumor cells in vivo was presented by T. C. Manning and D. M. Kranz. To specify minimal requirements for CTL induction, the authors generated mice with transgenic 2C TCR on $RAG^{0/0}$ background. In these mice all T cells were $CD8^+$ with identical specificity to $H\text{-}2L^d$ in the absence of other T and B cells. The transgenic mice efficiently rejected allogeneic P815 ($H\text{-}2^d$) cells even if huge amounts of these cells ($3x10^8$) were injected; however, transplantation of syngeneic EL4 ($H\text{-}2^b$) thymoma was lethal to these mice. Blocking of B7-CD28 costimulation with hybrid CTLA-4IgG3 protein did not prevent the induction of allospecific CTL and tumor rejection. Therefore, high incidence of precursors and high affinity allogeneic ligand for TCR allow to circumvent the dependence of the response on $CD4^+$ helpers and costimulation [139]. However, in mice with normal T cell repertoire the rejection of allogeneic tumor usually depends on costimulation [140]. Nevertheless, blockade of both pairs of major costimulatory ligands, B7/CD28 and CD40/CD154 (CD40L), allows to see the rejection of the cutaneous transplant independently of costimulation via direct recognition of allogeneic MHC molecules by $CD8^+$ cels (effect named "costimulation blockade resistant allograft rejection"). These $CD8^+$ cells independent of costimulation are induced only in responses to "strong" major histocompatibility antigens. If the donor and the recipient differ only in minor antigens, the blockade of costimulatory receptors leads to a prolonged survival of skin transplant [141]. D. Kreisel and B. Rosengard have demonstrated direct allogeneic recognition of vascular endothelium by $CD8^+$ cells using radiation chimerae reconstituted with $TAP^{0/0}$ bone marrow as recipients. In these animals the professional APC originated from bone marrow are incapable of cross-presentation, and this inability excludes indirect recognition. But the donor $TAP^{0/0}CD8^+$ cells develop properly in thymic microenvironment of recipient and can reject the cardiac allograft [142, 143].

The $CD4^+$ cells also can recognize the allogeneic MHC molecules directly. This was proven when the allogeneic cardiac allograft was engrafted into RAG knockouts. In these recipients the allogeneic heart transplants survive for an indefinite time. But if these recipients are injected with

purified $CD4^+$ cells, the transplant is rejected acutely. The rejection requires MHC class II molecules of the donor but not the recipient. Moreover, $CD4^+$ mediated rejection occurs in recipients with class II and RAG knockouts, i.e., in the full absence of recipient's T cells, B cells and MHC molecules that present the antigen indirectly [144]. Similar result was obtained after transplantation of epithelium of allogeneic embryonic thymus from the donor at a pre-circulation stage; such donor had no professional APC. The absence of MHC class II in recipients did not delay the rejection of the epithelial transplant even after $CD8^+$ depletion, i.e., when no known pathways of activation of naive T cells were functional [145]. D. Kreisel and B. Rosengard got different results using radiation chimerae reconstituted with the bone marrow from MHC class II knockouts. The goal of these experiments was to test, whether $CD4^+$ cells recognize the antigen directly. Unlike early data on $CD8^+$ cells, $CD4^+$ cells were incapable of direct activation by alloantigens presented by non-professional APC. The authors also analyzed the rejection of cardiac allografts from a) B6 chimerae reconstituted with the bone marrow of knockouts, and b) knockouts reconstituted with the bone marrow of B6 mice. In the first group MHC class II molecules were expressed only on endothelial cells but not on resident APC. In the second cohort all non-hematopoietic cells were devoid of MHC class II molecules whereas these molecules were found on the bone marrow derived resident APC. The rejection of transplants in the first group was delayed whereas it was rapid in the second group of animals. This indicated that the rate of heart transplant rejection was critically dependent on the direct recognition of MHC class II molecules presented on allogeneic bone marrow APC but not on other cells in the transplant [146].

Thus, the ***direct recognition of allogeneic MHC molecules is well documented and proven phenomenon. This mechanism is predominant in the responses to fully allogeneic graft. The $CD8^+$ activation and gain of effector functions can occur as a result of direct recognition of allogeneic MHC molecules on professional and non-professional APC whereas $CD4^+$ cells are activated as a result of direct recognition of alloantigens only on professional APC.***

Most likely, these differences between $CD4^+$ and $CD8^+$ cells can be explained by the fact that activation of $CD4^+$ cells requires coexpression of antigen specific and costimulatory ligand on the same APC [147]. The role of this coexpression is to create an immunological synapse between the T cell and APC [148, 149]. Dependence of $CD8^+$ on antigen presentation by professional APC is much less evident, because the activation of naive $CD8^+$ cells does not require organized synapse formation and supramolecular clustering [150, 151]. These facts allows to assume three-cell cooperation in which the $CD8^+$ cell recognizes the allogeneic MHC molecule on the surface of non-professional allogeneic APC, and the costimulatory ligand on the dendritic cell that does not present the antigen. This model has been provided repeatedly to counter the “two signals-one APC” dogma, and the mechanism of costimulation was termed “trans-costimulation” [152-155]. This model has explained how the $CD8^+$ response to non-professional allogeneic APC is induced being dependent on costimulation. Apparently, the model answers the question of why even the fibroblast transfected with LCMV genes becomes an efficient APC in the lymphoid microenvironment [156, 157].

9. Direct allogeneic recognition: peptides or side chains?

The hypothesis of initial priming explains alloreactivity as a consequence of innate preference of T cell receptors for recognizing MHC molecules of the species and/or higer density (or frequency) of allogeneic determinants presented by allogeneic MHC molecules.

The hypothesis of evolutionary preference of TCR genes according to the ability of their products to interact with MHC molecules of the same species was first provided by N. Jerne [15]. The hypothesis suggests that, after elimination of self-reactive T cells in the thymus the mature repertoire is comprised of high frequency of cells specific to all other MHC antigens. Since the selection in the thymus leads to enrichment of T cells capable of reacting with MHC, the pool of TCR genes would have been too abundant, and many “senseless” precursors would have been produced. This is why the more TCR gene products can react with MHC molecules, the more

specific is the thymic selection. Jerne's hypothesis is supported by some studies, although the efficacy of thymic selection is likely to be quite low [158, 159].

The hypothesis of "density of determinants" presumes differential expression of routine antigens presented by self MHC on self APC compared with the expression of allogeneic MHC molecules on allogeneic APC [160]. Accordingly, the density of allogeneic determinants expressed by self APC is very low because the majority of MHC molecules present the peptides unrelated to the alloantigen whereas the density of the determinants on the allogeneic APC is very high. The hypothesis states that there is no big number of alloreactive cells, but rather the allogeneic APC activate a lot of T cells due to a strong stimulus, and low affinity T cells become involved. Apparently, the flaws of this hypothesis are: 1) lack of convincing data on preferable involvement of low affinity clones in the allogeneic response; 2) no data on positive correlation between the intensity of the allogeneic response and the presentation of any particular peptides; and 3) the hypothesis provides no clue as to why T cell receptors recognize the antigens exactly in the context of MHC molecules.

The third explanation, i.e., the hypothesis of "frequency of determinants", is based on the dependence of MHC specific T cells on the peptides presented by these molecules. The recipient's MHC molecules present the peptides of self proteins to which the recipient develops tolerance. This autotolerance has nothing to do with the response to peptides presented by allogeneic APC. The hypothesis presumes that alloreactive cells in the recipient are abundant because each MHC alloantigen forms a variety of antigenic determinants [161]. Identification of different MHC binding motifs in the peptides that interact with allele specific forms of MHC molecules is a strong argument in support of this hypothesis. Obviously, these differences allow for presenting a broad spectrum of complexes of the peptides with allogeneic MHC molecules whereas none of these peptides is presented on APC of the responding organism. The disadvantage of this hypothesis is it does not explain the T cell recognition of antigens exactly in the context of MHC molecules.

The choice between the two latter hypotheses is dictated by the dependence of alloreactive T cells on the peptides presented by allogeneic MHC molecules. The alloreactive T cells can recognize the determinants independent of the bound peptide [162, 163]. Nevertheless, the bulk of data indicates that peptide independent recognition is rare phenomenon, and the alloreactive cells indeed recognize the allogeneic MHC molecules in an association with the peptides [92, 103, 164]. The dependence of alloreactive memory $CD8^+$ cells on the MHC molecule bound peptides has been found in our studies as well. The memory $CD8^+$ cells from B10.D2(R101) ($K^dI^dD^b$) obtained in the response of EL4 thymoma (K^bD^b) proliferate in MLR in response to heat shocked allogeneic stimulators from C57BL/6 (K^bD^b) wild type mice. The proliferation is abrogated if TAP knockout stimulators are used. So, direct recognition of H-$2K^b$ molecule on the allogeneic cell depends on the peptides bound to this molecule [134]. Interesting and convincing data on the role of peptides in alloreactivity were obtained in the "single MHC/peptide" system. The authors used transgenic pEa mice in which all class II MHC molecules are represented by the individual complex of A^b with AA 52-68 peptide of E_α, and DM-KO mice in which class II molecules are bound with the individual CLIP peptide from invariant chain Ii. The cited article demonstrated the important mechanisms of allogeneic and allorestricted recognition. First, both ligands appeared to be "poor" stimulators of the allogeneic response, supporting the hypothesis of "frequency of determinants" and pinpointing the presentation of diverse peptide repertoire for the induction of intense allogeneic response. Second, T cell hybridomas obtained in the allogeneic response to these "allorestricting complexes" were more sensitive to stimulation with the antigenic peptide; furthermore, these hybridomas recognized the peptide in a degenerated manner unlike the hybridomas from syngeneic "autorestricted" responders. Third, testing >500 alloreactive hybridomas showed that the majority of alloreactive T cells depend on the peptide but only 17% recognize this peptide specifically. The authors suggested that the peptides influence the allogeneic response by inducing weak conformational changes in α-helix of the MHC molecule, and these changes are recognized by alloreactive T cell receptors. The degenerated recognition of the peptide and high sensitivity to the peptide ligand are the key features of alloreactive T cells that regulate the strength of the response

[165]. Thus, ***the allogeneic response depends on the peptides bound to allogeneic MHC molecules. A significant percentage of clones activated by allogeneic MHC molecules specifically recognizes these peptides. This points at similarity of interaction of alloreactive and autorestricted T cells with their ligands, namely, MHC/peptide complexes.***

The side α-helices of MHC molecules also interact with TCR, and this interaction is important for alloreactivity. Cytotoxic T lymphocytes lyse the TAP negative allogeneic targets that express only low amounts of "empty" heavy chains of class I MHC molecules. Moreover, in bm3 TAP knockout mice $CD8^+$ cells are generated in the thymus and accumulate in the peripheral lymphoid organs. This indicates that positive selection can occur in the absence of bound peptides [73]. Singer and co-workers have demonstrated that allogeneic recognition of H-$2K^b$ molecule by cytotoxic T cells was blocked by the peptide of the same AA163-174 molecule, meaning there is a region for binding the heavy chain of MHC molecule with TCR [166, 167]. In our work the peptides from C terminal regions of α-helices devoid of MHC binding motifs for B10.D2(R101) recipients, when injected i.v., still induced cell mediated suppression of the allogeneic immune response and even extend the lifespan of the allogeneic skin graft in the recipients [168]. Another evidence of the interaction of TCR with fragments of MHC molecules was demonstrated with mutant MHC. Some individual point mutations in side α-helices of MHC molecules had no effect on the spectrum of bound peptides but cause an intense immune response [169, 170]. M. Pla and colleagues have shown that mutations in positions 62, 65, 69, 72, 152, 163 and 166 in α-helices away from peptide binding groove can be antigenic. The repertoire of peptides that bind individual mutants did not correlate with the ability of mutants to evoke primary immune response [171].

What amino acid residues are critical for recognition of class I MHC molecules by alloreactive and autorestricted CTL? Using large panels of alloreactive and autorestricted clones and targets that express mutated amino acid residues, it was shown that their recognition by alloreactive and autorestricted clones depended on the same residues in the heavy chains that form common clusters of recognition [172, 173]. The alanine mutagenesis based mapping of relative energies of interaction of TCR with with the MHC heavy chain revealed that approximately 2/3 of the surface and energy in the interface between the TCR and MHC molecule belonged to the interaction of the receptors with the heavy chain of class I histocompatibility molecule [174]. All these data indicate that ***alloreactivity cannot be explained solely by differences in the peptide repertoire presented by various MHC molecules. The side α-helices also play an important role being capable of direct interaction with the TCR.***

The MHC molecules present self and alien peptides to T cells. The groove MHC-peptide directed to the extracellular milieu and is a plane formed by α-helices of the MHC molecule and the peptide. Up to now the crystal structures of several TCR/MHC/peptide complexes are known. Topologically the interactions within each complex are similar to those in all other complexes. The TCR is oriented diagonally relative to the external surface of the MHC/peptide complex. Spaciously speaking, the CDR1 and CDR2 of α-chains of the TCR are localized near the N-end of the peptide whereas similar parts of the β-chain are positioned near the C terminus. The CDR1 and CDR2 chains encoded by V region interact mainly with amino acid residues of MHC molecules. The third region in the TCR is the most variable; this region determines the complementary interaction CDR3-MHC. The regions of CDR3α and CDR3β of the TCR are oriented to the center of the contact TCR/MHC/peptide and interact mainly with the central part of the peptide. Several residies in the peptide form the external surface of MHC/peptide complex and are available for the interaction with the TCR. This is why the most variable regions of CDR3α and CDR3β chains of the TCR have optimal access to the most variable component of the ligand, i.e., to the peptide [175]. Similar principle of TCR/MHC interaction was found for allospecific TCR Bm3.3 that binds class I H-$2K^b$ molecule complexed with naturally processed octapeptide (pBM1:INFDFNTI). In this complex the TCR and MHC bound peptide are linked via CDR3β region whereas in another TCRs α- and β-chains have equal impact on the interaction. Accordingly, only a few residues at the C terminus are involved in the interaction with the reeceptor. Another peculiarity of this complex is very small interface between the TCR and MHC, the smallest among the known surfaces of this

type. This is even more surprising since the affinity of interaction (determined by the method of plasmon resonance) was among the highest in this range. The CDR3α region of this TCR is large, it consists of 11 residues and is shifted from the peptide binding groove. This region interacts only with Gln65 of α-helix of α_1 domain of the MHC molecule whereas CDR3β consists of 9 residues that all interact with the peptide. The CDR1α and CDR2α are shifted to the N end of α-helix of α_2-domain of the MHC molecule, i.e., away from the peptide binding area, which abrogates their interaction with α-helices of the MHC molecule. In other words, the position of the ligand bound TCR is oblique and is mediated mainly by interactions with Vβ chain of the receptor with the side chain of the MHC molecule and C-terminal part of the peptide. These spacial considerations are relevant to degenerative mode of peptide recognition in the allogeneic response. For a particular TCR/MHC/peptide combination, predicted number of peptides interacting with Bm3.3 TCR can increase 400-fold [176]. ***The X-ray structure analysis is the most convincing proof of direct interaction of the TCR with the allogeneic MHC molecule. This analysis provided no significant difference in the topology of interaction of alloreactive and autorestricted TCR with MHC/peptide complexes. Variabilities in the length of CDR3 regions of Bm3.3. TCR may serve as structural basis for degenerative manner of recognition of the peptide ligand.***

However, evidence is growing in support of general character of degenerative recognition of MHC/peptide complexes by all TCRs. Usually a detailed analysis of cross-reactivity of individual TCRs reveals additional MHC molecules or peptide ligands capable of interacting with the receptors of interest (see section 4). Cross-reactivity was found in our studies of alloreactive MCC-1 clone of $CD8^+$ memory cells obtained in the response to allogeneic H-$2K^b$ molecule. This clone can be activated by the immunizing antigen as well as in the response to H-$2D^d$(L^d) and H-$2D^q$(L^q). However, the lengths of CDR3α and -β chains of its TCR are similar [134]. Most likely, degenerative manner of recognition is an important trait of the immune response that allows T cells to recognize enormously big variety of MHC bound peptides; the specificity is sufficient to discriminate between "self" and "foreign".

From the body of analyzed literature one can conclude that ***there are no critical differences in the recognition of the peptide in the context of syngeneic or allogeneic MHC molecule. Both types of interaction can induce highly specific immune response to individual MHC/peptide complexes. Both are promiscuous in recognizing other MHC/peptide combinations. Nevertheless, the response to allogeneic MHC/peptide complexes are characterized by a sizeable number of degeneratively recognizing clones that cross react with other MHC molecules by interacting with their α-helices.*** Supposedly, these promiscuous clones appear, because negative selection does not eliminate them.

10. Back to Jerne's hypothesis.

Is it reasonable to believe that the ability of T receptor repertoire to interact with MHC is genetically determined? Two independent groups have shown that non-selected T cell receptor repertoire has an innate capability to recognize MHC molecules in the thymus [158, 178]. The first group demonstrated that 20% of thymocytes recognize MHC molecules prior to positive and negative selection since CD69 activation marker was expressed on recognizing cells [178]. The second group used T cells from mice that do not express MHC molecules. These cells are DP lymphocytes with T cell receptor repertoire that was selected neither positively nor negatively on MHC molecules. As in normal mice, these cells are immature. Using monoclonal antibodies against TCRα/β and CD4 the authors efficiently induced the maturation of these lymphocytes in fetal thymic organ culture (FTOC). The analysis of TCR specificity revealed high frequency of clones that react with allogeneic MHC molecules in pre-selected T lymphocyte repertoire, and this frequency was similar to that after selection. This fact indicates the innate predisposition of the TCR to the interaction with MHC molecules. The genes coding for TCRs are evolutionary "designed" in a way their products bind predominantly the side α-helices of MHC molecules; this

is particularly true for CDR1 and CDR2 regions of V-segments [158].

These results shake the immunologists' worldview! Indeed, 1) preferential interaction of the TCR with the MHC molecule is not a consequence of positive selection in the thymus; 2) the T cell repertoire is primarily specific to all variants of classical MHC molecules, presuming a coordinated evolution of three independent genetic loci (α/δ, β and MHC); 3) alloreactivity and MHC restriction can be the sequelae of this innate specificity. The work by T. H. Yang and H. J. Stauss (2002) was logical development of the above works. The authors estimated four combinations of peptides with H-2K^b molecule to induce auto- and allorestricted responses in three murine strains. The responders were primed in vitro with stimulators loaded with peptides followed by evaluation of frequencies of peptide specific CTL. Three out of four peptides induced the responses restricted by self MHC better than by alien MHC, but the differences were only 3-5-fold. The fourth peptide induced auto-and allorestricted CTL with equal efficacy. Titration of peptides showed that high avidity CTL were present among auto- and allorestricted CTL. The authors concluded that ***narrowing the repertoire down to preferential recognition of antigens in the context of self MHC (which can be expected from positive selection in the thymus) is minor.*** Further analysis of lectin stimulated maturation of thymocytes from mice deficient in MHC expression showed that the number of K^b restricted CTL among these T cells is similar to the number of allorestricted CTL. Thus, ***MHC-restricted recognition of peptides is innate and immanent for T cell repertoire, and this recognition does not require thymic selection on MHC molecules*** [179].

What is the structural basis for genetic predisposition of antigen recognition by T cell? An exceptionally broad intraspecies polymorphism of MHC products allows for presenting maximal conceivable number of peptides. In placental mammals, the α_1 and α_2 domains of MHC class I molecules have equal length and contain 30% amino acid residues that are either invariant or carry silent mutations. High variability (more than 6 variants of substitutions) was found only in 38 out of 179 residues, and only in 9 cases out of 38 the substitution was localized to the peptide binding region whereas in 16 cases - to the TCR interacting region [180]. Still, hundreds of possible MHC haplotypes can be detected within the population. Such high polymorphism is not characteristic for the genes encoding Vα and Vβ segments of TCR. The primary structure of human and mouse TCRV subfamilies show high homology, and the homologous pairs of genes of a particular subfamily are closer each to other than the genes of different subfamilies. This points at trans-species evolution of TCR genes [181, 182]. Thus, ***despite the variability and multiplicity of MHC haplotypes and diversity of TCR due to TCR gene rearrangement, the TCR-MHC interaction can be genetically determined and mediated through conservative structures***. The functional result is narrowing of the repertoire of T cells with particular Vα and Vβ variants frequently observed in the allogeneic immune response [183-186]. This narrowing of the repertoire also occurs in the course of thymic selection. Some variants of Vα "prefer" to interact with certain class of MHC molecules. The T cells with such TCRVα are committed to CD8$^+$ differentiation to become reactive with class I MHC molecules or to CD4$^+$ differentiation (reactivity with class I MHC molecules). This innate feature is determined by CDR1 and CDR2 regions of Vα chain since point mutations in these regions can switch CD4$^+$/CD8$^+$ differentiation [187-189].

Importantly, N. Jerne postulated the genetic predisposition of the repertoire for MHC recognition as early as in 1971. His hypothesis states that "antibody specificity is determined by structural V-genes that code for the amino acid sequences of the variable regions of antibody polypeptide chains. The present hypothesis proposes that the germ-cells of an animal carry a set of V-genes determining the combining sites of antibodies directed against a complete set of certain class of histocompatibility antigens of the species to which this animal belongs. The evolutionary development of this set of V-genes in phylogeny is traced back to the requirements for cell to cell recognition in all metazoa. The hypothesis leads to a distinction between two populations of antigen-sensitive cells. One population consists of cells forming antibodies against foreign antigens; these lymphocytes have arisen as mutants in clones descending from lymphocytic stem cells which expressed V-genes belonging to the subset (subset S) coding for antibody against histocompatibility antigens that the individual happens to possess. The other population consists of

allograft rejecting lymphocytes that express V-genes of the remaining subset (subset A) coding for antibody against histocompatibility antigens of the species that the individual does not possess. The primary lymphoid organs are viewed as mutant-breeding organs. In these organs (e.g. in the thymus), the proliferation of lymphocytes expressing the V-genes of subset S and the subsequent suppression of the cells of these "forbidden" clones, leads to the selection of mutants cells expressing V-genes that have been modified by spontaneous random somatic mutation. This process generates self-tolerance as well as a diverse population of antigen-sensitive cells that reflects antibody diversity. The proliferation in the primary lymphoid organs of lymphocytes expressing V-genes of subset A generates the antigen-sensitive cell population that is responsible for allo-aggression. The theory explains how a functional immune system can develop through a selection pressure exerted by self-antigens, starting during a period in early ontogeny that precedes clonal selection by foreign antigens. The hypothesis provides explanations for the variability of the N-terminal regions of antibody polypeptide chains, for the dominant genetic control of specific immune responsiveness by histocompatibility alleles, for the relative preponderance of antigen-sensitive cells directed against allogeneic histocompatibility antigens, for antibody-idiotypes, for allelic exclusion, for the precommitment of any given antigen-sensitive lymphocyte to form antibodies of only one molecular species and for the cellular dynamics in the primary lymphoid tissues."

Although this hypothesis is more than 30 years old, its importance remains astounding in our days.

11. Questions for discussion, or "What is going really"?

Most likely, the amount of data that do not fit the hypothesis of ontogenetic origin of MHC restriction is overwhelming. The experimental approaches on which this hypothesis was founded were criticized by the pioneer of MHC restriction. The results on the animals with transgenic TCR are largely refuted. It is accepted that the specificity of restriction by T cells is associated not with the thymus but with cells originated from bone marrow with minor impacts of microenvironment and survival at the periphery. As a result of the drift of ideas, the hypothesis of "initial priming" emerged that states the critical role of the primary immune response to a certain MHC/peptide combination. Due to this priming a portion of T cell repertoire arises, that is specific to recognition of particular complexes MHC/peptide. In other words, the specificity is dictated by the antigen presenting cell. Limiting this specificity by thymic "bringing-up" on individual MHC/peptide complexes formed variable repertoire of T cells capable to react with allogeneic MHC molecules. Identification of MHC-binding motifs in antigenic peptides provided a substantial structural basis for explanation of the orgin of MHC restriction in terms of "initial priming" hypothesis. The combinatory peptide libraries proved the existence of broad repertoire of allorestricted T cells and revealed quite insignificant role of positive selection on narrowing the repertoire down to autorestricted clones. Taking into account that adaptive differentiation can not provide some explanations to phenomena of allorestricted responses and direct allogeneic recognition, it is time to admit that the ontogenic origin of MHC restriction, a hypothesis predominant in all modern immunological textbooks, is misleading.

This hypothesis tries to explain alloreactivity as cross-reactivity with "self". But what is a reason for these "cross-reactive" alloreactive clones not to be deleted during intrathymic negative selection? Are cross reactions more pronounced than specific reactions? The most obvious answer is no. Common sense suggests all the way around: ***the reaction of the repertoire to foreign transplantation antigens is specific whereas the recognition of peptides in the context of self MHC molecules is a "cross reaction". This presumes the initially predetermined specificity to all allelic products of the species' MHC, and the repertoire can recognize any such product as foreign prior to thymic selection. Negative selection eliminates the clones specific to "self" and leaves all others, among which are autorestricted, allorestricted and cross reacting in the alien MHC environment. Encounter of modified "self", i.e., alien or mutated endogenous peptide***

presented by self MHC molecule triggers the response of a portion of clones that are initially specific to allogeneic MHC molecules. This response is a cross reaction of the repertoire to the pathogenic peptide plus self MHC molecule. In turn, this implies that genetic polymorphism of the species' MHC molecules reflects (perhaps incompletely) the spectrum of specificities of TCRs. In that sense negative selection serves for adapting the pre-selected monomorphic repertoire of T cells to transplantation antigens of the organism to avoid the transplantation conflict with self. Loss of some specificities potentially useful for responses to pathogens is inevitable. Such loss makes Achille's heel in the organism attacked by pathogenic bacteria or tumors. Because the MHC molecules are highly polymorphic, and because their allelic forms present different peptides from the same protein, this vulnerability is individual within the same species. This is why selection of virus variants for the ability to escape the immunological attack in one organism would not rescue this variant from the immune system of different host. Therefore, polymorphism of MHC makes an "safety-net" that allows slowly evolutioning vertebrata to survive in the presence of quickly evolutioning pathogenic organisms.

Immunologists used to consider the traits of the immune system in the context of its co-evolution with pathogenic microorganisms. From this viewpoint polymorphism of MHC molecules would mean the variability of only those amino acid residues that are responsible for presentation of peptides. However, the majority of variable residues are localized in TCR binding regions and have no impact on specificity of peptide binding; nevertheless, these variable residues are necessary for alloantigenicity of MHC molecules. The reason for this polymorphism is unclear considering the traditional role of MHC, namely, antigen presentation. This reason can be grasped based on our hypothesis that pinpoints alloantigenic traits of MHC molecules. Indeed, changes in alloantigenic "image" of the molecule would change the character of negative selection of the repertoire; in turn, this would provide an opportunity to rescue individual clones that respond to the pathogen. We estimated the variability of amino acid residues of mammalian class I MHC. After we found the motifs and the spectrum of individual substitutions, the limits of amino acid variability within the groove became obvious. The probability of difference between determined structural motif and individual substitution was 0.9% whereas for TCR binding residues it was 5-fold higher (4.3%). This suggests that evolution exhausted the variants of groove forming residues but the interface between TCR and MHC is still changing [Kazansky D.B. Motifs in primary structure of mammalian MHC molecules. (In Russian) http://kazansky1.narod.ru/works/motifs.html]. This fact is also difficult to explain if to exclude the ability of TCR to directly interact with MHC molecules, the alloantigenicity of MHC and its role in repertoire formation.

The proposed hypothesis seems to reconsile the experience of transplantation immunology with the MHC restriction concept and to explain alloreactivity. The hypothesis provides one general foundation for these phenomena, i.e., interaction of the repertoire with histocompatibility molecules. The hypothesis of adaptive differentiation failed to explain alloreactivity, and even genetic control of allogeneic reactions and MHC restricted recognition was considered differentially. The ability to induce the specific allogeneic response is inherited as a dominant trait whereas the ability to induce MHC restricted responses - as a co-dominant one. To the reseachers of MHC restricted recognition the allogeneic effects were distracting; genetically defined animal strains were needed for an adequate experimental exploration to avoide these effects. So it is understandable that allogeneic phenomena were out aside for the time being. The situation changed after the discovery of allorestricted recognition. Jerne's hypothesis solved the enigma of dominant inheritance of alloantigenicity by linking the structure of V genes and the innate predisposition of the repertoire to recognize transplantation antigens. Astonishingly precise provision of Jerne was confirmed by the experiments of Jens Zerrahn and David Raulet. The discovery of peptide presentation by MHC molecules showed that allelic forms of MHC molecules present different peptides. J.-P. Kovalick and L. van Kaer demonstrated an important role of a broad peptide repertoire in the intensity of the allogeneic response. Altogether, our hypothesis states that ***high intensity of the allogeneic response is explained by two non-exclusive peculiarities of T cell recognition: 1) genetically determined ability of the repertoire to interact with the entire***

spectrum of the species' MHC molecules, and 2) specificity of negative selection that maintains the ability of the repertoire to respond to peptides in the context of allogeneic MHC molecules.

Apparently, the most important problem for the immune system is to avoid transplantation conflict within the organism. Indeed, the receptors of adaptive immunity "see" self antigens much more frequently than pathogenic bacteria. In any case, an existence of potentially dangerous system in the organism could be not less important factor for the evolution of immune system than pathogenic microorganisms. Therefore, ***MHC restricted recognition could develop not as much in the struggle with pathogenic microorganisms but in inhibiting the reactions with "self". On this way, restriction of immune reactions by several types of recognized molecules would be helpful, because rescued another biological macromolecules from danger. MHC molecules have allowed to focus these reactions on short peptides containing aminoacid substitutions not presented in responding organism. On the other hand, they have allowed efficient formation of specific central tolerance to self not involving in this process a huge diversity of another protein molecules. So, alloreactivity can be not only a back side of efficiency in induction of central tolerance, but the general feature of T cell repertoire.*** Moreover, the interactions with transplantation antigens can be a basis for building adaptive T cell mediated immunity. This notion is partly confirmed by the observation that central tolerance to self is MHC-restricted. The CTL 27.B2 clone obtained in response of bm1 mice to B6 stimulators recognized the same peptide in the context of allogeneic H-2K^{b} molecule but not syngeneic H-2K^{bm1} [169]. Also, similarity of genetic control of MHC restriction and central tolerance to self, both inherited co-dominantly, supports the above considerations.

One argument of the adepts of adaptive differentiation is an artificial, purely laboratory character of alloreactivity, phenomenon that does not exist in natural situations. Nevertheless, the reaction of thymocytes to self MHC during intrathymic selection has much in common with the reaction of the mature repertoire to transplantation antigens. Both reactions depend on the same co-stimulatory ligands and co-receptors [190, 191]. Both depend on avidity of T cell-APC interaction, although the threshold of activation for thymocytes seems to be lower than that of mature peripheral T lymphocytes [192, 193]. Clearly, positive selection can be an analog of peripheral interactions of T cells with self MHC necessary for survival [194]. Negative selection in the thymus is evidently similar with deletion of peripheral T cells upon high affinity binding of endogenous superantigens [195]. A significant difference is that the interaction of thymocytes with self MHC leads to deletion of thymocytes whereas the mature T cells gain the effector functions after the reaction to alloantigens [196]. Thus, ***thymic selection can be considered as "first and last" a life-long allogeneic reaction of pre-selected repertoire to self transplantation antigens***. This process solves two problems: 1) to delete autoimmune and cross-reactive (promiscuous) clones that react with self transplantation antigens, and 2) to keep the repertoire of specificities to all conceivable pathogens as broad as possible. The first problem is resolved by negative selection in the thymus and deletion of autoimmune and cross-reactive clones. The second task is accomplished via preservation of a portion of T cell repertoire through weak degenerated interaction with self transplantation antigens. Since the repertoire is specific to the whole spectrum of the species' MHC molecules, the remaining portion of the repertoire would contain the clones that might be "autoimmune" or "cross-reactive" in different MHC environment. This provides good explanation for frequent cross-reactivity of clones rised in the allogeneic response, and provides the reason for degenerated mode of recognition of alien antigens and allelic MHC products by TCRs. Definitely, the degenerative fashion of recognition is necessary for preserving a broad spectrum of specificity of the repertoire to yet unencountered pathogens, given that this specificity is innate to all transplantation antigens of the species.

The main goal of this review was to bring out inner conflicts in modern theories of MHC restriction and allogeneic recognition, and to present an alternative concept that reconciles the contradicting viewpoints. Some considerations expressed by the author have been initiated by the idea of Jerne, the discovery of Jens Zerrahn and David Raulet and subsequent works [15, 158, 194, 197-200].

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