Nature of the minor histocompatibility antigens

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Human bone marrow transplants performed as therapeutical treatment of severe aplastic anaemia, leukaemia and immune deficiency disease became available in the seventies. For the present, the long-term results of allogeneic bone marrow transplantation (BMT) have greatly improved due to the use of HLA-matched siblings as marrow donors, advanced pretransplant chemoradiotherapy, the use of potent immunosuppressive drugs as Graft-versus-Host-Disease (GvHD) prophylaxis, better antibiotics and isolation procedures. Nonetheless, the results of clinical BMT reveal that the selection of MHC identical donors/recipients is not a guarantee of avoidance of GvHD or disease free survival even when donor and recipient are closely related. Allogeneic BMT especially in adults results, depending on the amount of T cell depletion of the graft, in uptil 80% of the cases in GvHD. In the HLA genotypically identical situation it amounts to 15-35% whereas in the phenotypical HLA matched recipient/donor combinations, the occurrence of GvHD is significantly higher *i.e.* 50-80% [1]. It is believed that disparities for minor histocompatibility antigens (mHag) between donor and recipient constitute a potential risk for GvHD or graft failure, which necessitate life long pharmacologic immunosuppression of organ and bone marrow transplant recipients. mHag can evoke strong MHC restricted cytotoxic (CTL) and proliferative T (Th) cell responses which can be measured *in vitro* [2]. Our laboratory has a series of well defined CTL and Th cell clones which are specific for sexlinked and non-sexlinked mHag, all of them are recognized in a classical MHC restricted fashion.

Functional studies

We studied the male specific mHag H-Y and five non-sexlinked mHag (designated HA-1 to HA-5) at three levels *i.e.* immunogenetics, immunogenicity and tissue distribution (as reviewed in [2]).

Immunogenetics

Immunogenetic studies revealed that some mHag appeared frequent (69-95%), others occurred with lesser frequencies (7-16%) in the healthy population. An analysis of the genetic traits demonstrated a Mendelian mode of inheritance independent of HLA.

Immunogenicity

Three sets of data are indicative for a hierarchy in immunogenicity among mHag. Firstly, CTL clones reactive to the same mHag HA-1 were obtained from peripheral blood lymphocytes of 3 out of 5 individuals each transplanted across a multiple and probably distinct mH barrier. Secondly, recent results indicate that the latter HA-1 specific CTL clones derived from different individuals, all seemed to use an identical TCR Vβ, showed remarkable similarities within the N-D-N regions, but used distinct Vα and γ segments. Thirdly, in a retrospective study, comprising 148 bone marrow donor/recipient pairs, investigating the influence of mHag HA-1 to HA-5 mismatching on the development of GvHD, we observed a significant correlation ($P = 0.02$) between mHag HA-1 mismatch and GvHD [3].

Tissue distribution

Tissue distribution studies revealed differential expression: some mHag (*i.e.* H-Y, HA-3 and HA-4) are expressed on haematopoietic as well as on non-haematopoietic cells, while the expression of other mHag (HA-1 and HA-2) is limited to cells of the haematopoietic lineage only. Moreover, all mHag are present on clonogenic leukemic precursor cells [4] as well as on circulating leukemic cells of lymphocytic and myeloid origin [5].

We may conclude from the results of the clinical related studies that mHag are involved in both Graft-versus-Host and Graft-versus-Leukemia activities

Molecular nature of minor histocompatibility antigens

Using immuno and biochemical isolation and purification procedures combined with sensitization of target cells for T cell recognition, mHag peptide containing fractions responsible for target cell sensitization were identified Subsequent mass spectometric analysis determined the amino acid (AA) sequence of to our knowledge the first mHag, *i e* HA-2, identified Synthetic peptides generated accordingly sensitized an HA-2 negative target cell to lysis by the mHag HA-2 specific CTL and herewith confirmed the correct mHag HA-2 AA sequence This peptide most probably originates from a member of the non-filament-forming class I myosin family [6]

Another mHag we recently identified is the male specific antigen H-Y [7], an antigen searched for since 1955 The first report on H-Y as a transplantation antigen is an untitled communication by Eichwald and Silmser These authors observed that within two inbred strains of mice, most of the male-to-female skin grafts were rejected, whereas transplants made in other sex combinations nearly always succeeded [8] In the human situation the first report on involvement of H-Y in transplantation appeared in 1976 [9] It concerned a clinical observation of a bone marrow graft rejection of a male HLA identical sibling donor by his HLA identical sister *In vitro* analysis of the posttransplant peripheral blood lymphocytes (PBLs) of this female patient (HLA phenotype HLA-A2, -A2, -B44, -B60, -Cw3, -Cw5, -DR4, -DRw6) showed unambiguously strong CTL responses specific for male HLA-A2 positive target cells Besides, this study also evidenced the first demonstration in man of the classical phenomenon of MHC restricted recognition of foreign antigen [9]

Sensitization to the H-Y antigen extends to organ transplantation, bloodtransfusion and possibly also pregnancy wherein MHC restricted T cell responses to the mHag H-Y in association with different MHC molecules are observed (reviewed in [2])

The same isolation and identification techniques as applied for the characterization of mHag HA-2 (*described above*) was used to identify the human mHag H-Y The H-Y antigen presented by HLA-B7 appears an 11-residue peptide derived from SMCY, an evolutionarily conserved protein encoded by the Y chromosome [7, 10] The human Y gene coding for the mHag H-Y is possibly functioning as a gene controlling spermatogenesis We are currently characterizing the mHag HA-1 as well as H-Y T cell epitopes eluted from other HLA molecules

Summarizing, mHag are peptides from polymorphic self proteins They are derived from evolutionarily conserved genes with important biological function

Clinical applications

As more mHags become biochemically identified, molecular typing can now be used for diagnostic in bone marrow donor selection Dissection of the major from the minor minors and their biochemical identification may aid in immunomodulatory approaches Designing mHag peptide analogues which function as MHC or T cell receptor antagonists might interfere with the harmful anti-host T cell reactivities post HLA identical BMT The potential application of mHag with broad tissue distribution like H-Y and HA-3 may aid in induction of tolerance in mHag negative bone marrow donors to prevent GvHD and in mHag negative bone marrow recipients to prevent rejection The mHag H-Y peptide information may also be used in the generation of genetic probes to be used for prenatal diagnosis in sex-linked congenital abnormalities and investigating minimal residual disease and chimerism Most promising is immunotherapy using CTLs specific for mHag peptide for the treatment of refractory, residual or relapsed leukemia The mHag with restricted tissue distribution (*e g* HA-1 and HA-2) are candidates for adoptive immunotherapy of leukemia Upon transfusion, the mHag peptide specific CTLs will kill the haematopoietic cells including the leukemia cells and will spare non-haematopoietic cells

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