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Minor H antigens—a "1993" look into their perspectives

Els Goulmy, Dept. of Immunohaematology and Blood Bank, University Hospital of Leiden, Rijnsburgerweg 10, 2333 AA Leiden, The Netherlands.

Bone Marrow Transplantation (BMT) in combination with chemoradiotherapy is a general treatment of severe aplastic anemia, leukemia and other hematologic malignancies. Despite advances in pre-BMT conditioning, better Graft-versus-Host-Disease(GvHD) prophylaxis and HLA matching, allogeneic BMT espacially in adults has, depending on the amount of T cell depletion of the graft, uptil 80% of the cases GvHD. In the genotypically identical situation it amounts to 15-35% whereas in the phenotypical HLA matched patient/donor combinations, the occurrence of GvHD is significanthy higher i.e. 50-80% (1,2). Differences between BM donor and recipient for as yet unknown MHC coded transplantation antigens as for non-MHC coded (the so-called minor Histocompatibility(mH) antigens) form important transplantation barrieres. The latter antigens play a role in the development of GvHD in the HLA genotypically identical combinations. In situations where an HLA phenotypical matched donor is used, not only mH antigens but also HLA variants (3) and as yet unidentified MHC antigens can induce GvHD.

This brief review will focus on some new aspects and insights into the functional characteristics of human mH antigens. The last few years evidence has accumulated that in addition to cytotoxic T lymphocytes (CTLs), mH antigen specific T helper (Th) cells could be relevant in the pathogenesis of GvHD. Mouse strains have been identified in which the L3T4+subset alone induced GvHD, although the additional presence of Lyt2+ cells in the donor inoculum intensified the GvHD reactions (4). In man, the presence of a reduced number of T4+ve cells in the donor marrow inoculum appeared compatible with slow but sustained engraftment and a low incidence of serious acute GvHD (5). In vitro studies reporting on host directed proliferative T cells have only scarcely been described in patients having GvHD (6,7,8). Van Els et al. reported on the long term kinetics of Th cells in response to host mH antigens in 16 patients, and demonstrated that significant Th cell activity in vitro correlates with clinical aGvHD (9). In a subsequent study, we demonstrated that these anti-host Th cells carry the CD4 phenotype and recognize mH antigens in the context of HLA-DR and -DP (10).

Some of these mH antigen specific Th populations may have the potential to serve as effector cells at the local site of inflammation. Recent work by our group demonstrated that MHC class II+ve, ICAM-1+ve keratinocytes have the potential to activate mH antigen specific Th cells in vitro (11). So, apart from a tolerizing effect ascribed to this non professional antigen presenting cell (12), interaction of primed antigen specific Th cells with activated keratinocytes may also result in enhancement of a cutaneous immune response in vivo. Not all mH antigen specific Th cell clones could be activated in vitro by keratinocytes. In our study the absence of expression of a mH antigenic epitope on keratinocytes. This supposition is plausible since mH antigens, defined by GvHD derived CTLs, show variable expression on human skin cells (13).

Most recent observations support the notion that mH antigen specific Th cells are by and large likely to play a role in the pathogenesis of acute GvHD. A limited dilution assay was developed to measure the frequency of pretransplant donor Th cell precursors against host mH antigens. Preliminary results in donors of HLA-genotypically identical BM indicated that a high frequency of helper T cells might be predictive of subsequent severe acute GvHD (14, 15). Whereas in both latter studies the primary in vitro mH antigen Th activities have been measured by IL-2 production of the responding cell population, we recently focussed on the use of a more professional antigen presenting cell as stimulating cell in order to induce primary mH antigen Th cell responses in vitro. Indeed, our preliminary results demonstrated the capacity of human Dendritic Cells to induce mH T cell activities in a primary Mixed Lymphocyte Dendritic Cell Reaction (MLDCR) between HLA genotypically identical sibling pairs (Van Lochem submitted). The first series of experiments showed variability in the MLDCR stimulation indexes(varying from 15 to 35) within and between the HLA identical pairs. These results would be indicative for a hierarchy in immunogenicity among the human mH antigens. Assuming that as in the mouse (16) the human genome has an abundancy of mH loci, some recent results by our group are suggestive for the existence of immunodominant mH antigens. From peripheral blood lymphocytes of 3 individuals each transplanted across a multiple (and probably distinct) mH barrier, CTL clones reactive to the same antigen termed mH HA-1 were obtained (17). Along with the latter observation an ongoing retrospective analysis, comprising 150 HLA sibling donor/recipient pairs, investigating the influence of mH antigens HA-1 to HA-5 on the development of GvHD sofar indicates a prominent role of mH antigen HA-1(manuscript in prep.).

Another area of interest is the possible involvement of mH antigens in the so called Graft-versus-Leukemia effect which according to clinical data parallels acute and chronic GvHD (18,19). In an attempt to study the post BMT anti-host CTL responses for their putative anti-leukemic activity in vitro, we observed "GvHD" related and "GvL" related activities.The latter type of CTL clones recognized patient's neoplastic cells only. The former type of CTL clones were reactive with ligands, like mH antigens, shared by host peripheral blood lymphocytes and leukemic cells (20). Another line of investigation support the notion that anti-host mH antigen specific CTL may play a role in the anti-leukemic effect of allogeneic BMT. Namely, mH antigen specific CTLs are capable of inhibiting in vitro outgrouth of clonogeneic leukemic precursor cells as well as lyse freshly obtained myeloid and lymphoid leukemic cells (21,22).

Finally, new insights into the nature of mH antigens has recently become available. Naturally, an answer to the question what mH antigens are is really needed. It would not only reveal their physiological nature but more importantly

provide inside into their putative role in organ and bone marrow transplantation. We therefore recently aimed at the biochemical characterisation of human mH antigens. Hereto, we made use of the immunopurification and biochemical techniques succesfully applied by Rammensee and his colleques to extract murine mH peptides from MHC molecules. Indeed, HPLC seperation of low Mr molecules (< 10 kD) obtained from acid treated MHC class I HLA-A2.1 molecules appeared successful. Fractions with sensitizing activity for the non-sexlinked mH antigen HA-2 specific CTL clones were isolated (23). Our observations are in line with previous reports on the isolation of naturally occurring peptides that represented classical murine mH antigens i.e. H-Y and H-4 (24,25). Similar to our own results, Sekimata et al. isolated an human mH antigenic peptide from EBV-LCLs by acid elution. This HLA-B35 restricted mH antigen was earlier shown to play a role in HLA identical kidney graft rejection (26). Further characterization i.e. exact amino acid sequence and identity of the protein from which murine or human mH antigens originate remain to be determined.

Little is known thus far on the molecular mechanisms involved in the intracellular generation of MHC class I restricted mH peptides. The prediction that they are naturally processed fragments of intracellular proteins that associated with MHC products could recently be verified. The availibility of a human cell line lacking both transport and proteasome subunit genes enable us to study the processing and presentation of human mH antigens. We demonstrated that the transport gene products were required for processing and presentation of antigenic peptides from influenza virus and from the intracellular mH protein HA-2 (27).

In conclusion, although lots of information was gathered during the past decades on the murine and human mH antigens, still many questions remain to be answered. Besides identification of the mH antigens and the genes they are encoded by, we must be able to dissect the majors from the minor minors. To achieve this, more information is needed on the Th and CTL defined human mH antigens repertoire, and to establish the immunodominant ones. To understand their biological role in bone marrow transplantation information on their cytokine secretion profile is essential.

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