The human minor histocompatibility antigen HA-1 as target for stem cell based immunotherapy of cancer : pre-clinical and clinical studies

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Chapter 5.

Chromosomal aberrations in leukaemia cells may delete tumour target antigens of stem cell-based immunotherapy

Chromosomal aberrations in leukaemia cells may delete tumour target antigens of stem cell based immunotherapy

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A novel approach to eliminate residual disease after human leukocyte antigen (HLA)-matched stem cell transplantation (SCT) for leukaemia is tumour specific adoptive immunotherapy. Excellent immunotherapeutic targets for adoptive immunotherapy are the haematopoietic-system specific minor-histocompatibility antigens (mHags) HA-1 and HA-2, which are expressed on all normal and malignant haematopoietic cells (1;2). HA-1 and HA-2 epitopes are presented on the cell surface in the binding groove of HLA-A2 molecules. Immunotherapy with HA-1 or HA-2 specific cytotoxic T lymphocytes (CTLs) is restricted to leukaemia patients positive for the immunogenic mHag HA-1 or HA-2 alleles, i.e. HA-1H or HA-2V respectively. The mHag status of patients is routinely determined by allele-specific genomic polymerase chain reaction (PCR) on peripheral blood mononuclear cells (PBMCs) (2). Generally, leukaemic cells have the same mHag allelic patterns as the PBMCs. Most leukaemias however have karyotypic abnormalities (3), some of which affect mHag encoding genomic regions (e.g. 19p13.3 harbouring the HA-1 gene (4) or 7p12-13 harbouring the HA-2 gene (5)).
Here, we describe an isochromosome 7 causing loss of HA-2 CTL recognition of leukaemic cells in a patient with pre-B acute lymphoblastic leukaemia. The patient was typed on PBMCs to be heterozygous for both HA-1 (H/R) and HA-2 (V/M) (Figure 1e,f). The leukaemia karyotype was 46,XX,i(7)(q10),der(19)t(1;19)(q23;p13). Hereby, the HA-1 encoding region on one chromosome 19 and the HA-2 encoding region on one chromosome 7 were deleted (Figure 1a). The bone marrow of the patient contained 90% leukaemic blasts. We separated these CD10 positive leukaemia cells from non-leukaemic cells with magnetic beads and used them as targets in a chromium release assay. HA-1 CTLs effectively lysed the isolated leukaemic cells and a leukaemia cell line derived from the same patient (Figure 1b). In contrast, HA-2 CTLs did not lyse the leukaemia cells. Phytohemagglutinin blasts of the patient and the CD10 negative bone marrow fraction were however well recognised by HA-2 CTLs. Low recognition of unseparated bone marrow cells is explained by the high percentage of HA-2 non-expressing leukaemia cells (Figure 1c). We subsequently repeated genomic mHag HA-1 and HA-2 typing on highly purified leukaemia cells (Figure 1d-f). Both, HA-1R and HA-2V were absent in the leukaemia cells, while present in PBMCs and the non-leukaemic bone marrow fraction. Thus, the chromosomal aberrations had deleted the non-immunogenic HA-1R allele and the immunogenic HA-2V allele, concordant with the functional results. Here, we have shown as proof of principle that common karyotypic abnormalities in leukaemia cells can knock out mHag encoding genes and thereby abrogate their recognition by mHag CTLs. This antigen presentation defect is restricted to a very limited number of antigens, namely those affected by the chromosomal aberration. It differs thereby from previously described immune escape mechanisms like loss/down-regulation of HLA-expression (6) or peptide processing defects (7) which broadly abolish antigen recognition on malignant cells. Consequently, the observed mechanism of mHag loss requires particular attention when targeting specific mHags with immunotherapy.

Chromosomal aberrations affecting mHag encoding genes are critical for mHag specific leukaemia recognition mainly in mHag heterozygous patients ("mHag heterozygous" means generally, that these

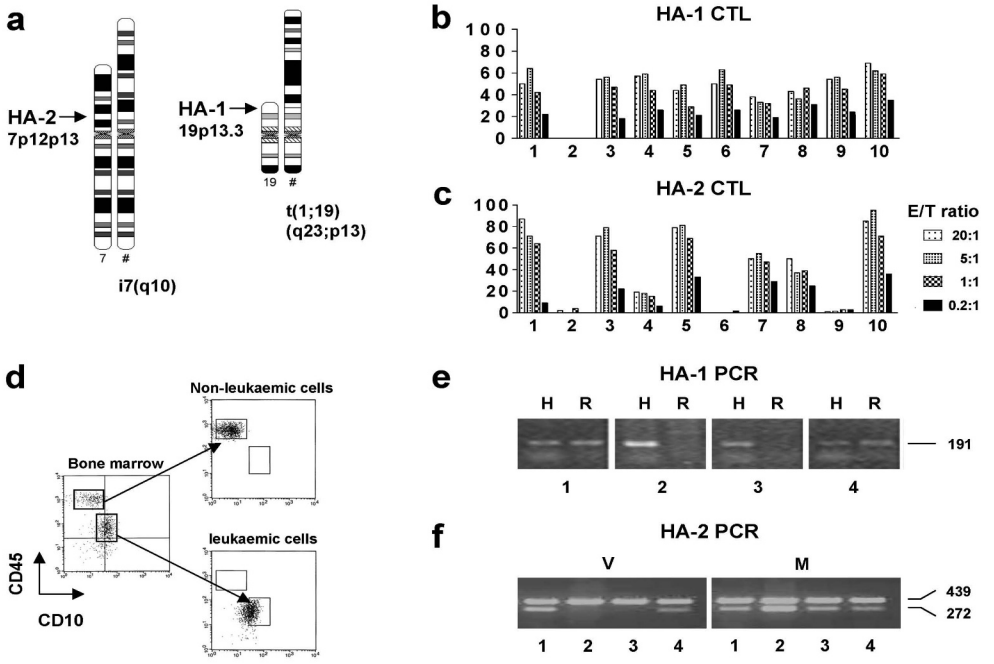

Figure 1. Chromosomal, mHag allelic and functional characterisation of the studied leukaemia. (a) Indicated are the normal chromosomes 7 and 19 in comparison to their aberrant counterparts (indicated with „#") in the leukaemia cells. Isochromosome 7(q10) means replacement of the genetic information on the p-arm (including the HA-2 locus on 7p12p13) by a duplicated q-arm. The unbalanced translocation t(1;19)(q23;p13) is associated with loss of HA-1 on 19p13. (b, c) Cytotoxicity of an HA-1 CTL clone (b) and of an HA-2 CTL clone (c) against different target cells were determined in a chromium release assay. x-axis, different targets (1-10) in different effector-target cell (E/T) ratios; y-axis, percent specific lysis. Target cells: 1: Epstein-Barr virus-/lymphoid cell line (EBV-LCL) from an HA-1H/R and an HA-2V/V donor, 2: EBV-LCL from an HA-1R/R and an HA-2M/M donor, 3: Phytohemagglutinin blasts of the patient, 4: unseparated leukaemic bone marrow cells, 5: unseparated leukaemic bone marrow cells loaded with HA-2 peptide, 6: primary leukaemia cells sorted for CD10 with magnetic beads, 7: primary leukaemia cells sorted for CD10 loaded with HA-2 peptide, 8: CD10 negative bone marrow cells, 9: leukaemia cell line of the same patient, 10: leukaemia cell line of the same patient loaded with HA-2 peptide. (d) Separation of $CD10^{high}$, $CD45^{dim}$ staining leukaemia cells from CD10 negative, $CD45^{high}$ staining non-leukaemic cells from leukaemic bone marrow (left scatter plot). Cell separation was performed by flowcytometry. Purity of the sorted populations is shown in the right scatter plots. (e, f) Allele specific PCR for HA-1H and HA-1R (e) and HA-2V and HA-2M (f). In the HA-2 PCR a control band is visible above the specific amplification product. Samples: 1: DNA from patient's PBMCs, 2: leukaemia cell line of the patient, 3: primary leukaemia sorted by flowcytometry, 4: non-leukaemic cells sorted by flowcytometry.

patients have one immunogenic and one non-immunogenic mHag allele). In these patients, loss of the single immunogenic mHag allele may completely abrogate leukaemia recognition by mHag CTLs. The risk of mHag HA-1 or HA-2 loss in haematological malignancies can be assessed as follows: Due to the restriction of the HA-1 and HA-2 epitopes to HLA-A2, only HLA-A2 positive patients can be affected by a loss of leukaemia recognition by HA-1 or HA-2 CTLs. Aberrations on chromosome 19 (harbouring

the HA-1 gene) and 7 (harbouring the HA-2 gene) mainly occur in childhood acute lymphoblastic leukaemia and secondary myeloid leukaemia, respectively (3). The risk of mHag HA-1 or HA-2 gene loss is thus largely restricted to these haematological malignancies. Approximately 50% of the individuals are HA-1 H/R heterozygous and 35% are HA-2 V/M heterozygous (8). This means that both HA-1 and HA-2 heterozygosity are frequent in the population. Therefore, HLA-A2 positive leukaemias with HA-1 or HA-2 gene affecting karyotypes might be occasionally unrecognized by HA-1 or HA-2 CTLs.

In conclusion, mHag loss due to chromosomal aberrations has relevant implications for the eligibility of patients for mHag specific immunotherapy. Confirmation of the mHag genotype on purified leukaemia cells is essential in mHag heterozygous patients with a leukaemia karyotype involving the mHag encoding gene.

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