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Clinical relevance of soluble HLA and interaction of papain derived class I molecules with alloreactive CTL

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Abstract. MHC class I and class II molecules are expressed in soluble form in the serum of both healthy and diseased individuals. Our aim was to investigate whether soluble class I (sHLA) levels in allograft patients correlate with their clinical status. Altogether, 20 renal and 30 cardiac graft recipients were examined. High levels of sHLA were measured at least 5 days preceding acute rejection episodes. Immune complexes between anti-HLA antibodies and sHLA were detected in a patient who died of a severe vascular rejection. In another study the interaction of papain-derived sHLA on alloreactive CTL in vitro was investigated. In a chromium-51-release cytotoxicity assay, 1,25 μg/ml of papain-digested class I molecules reduced CTL cytotoxicity to background levels. On the contrary, immobilized molecules triggered the release of serine esterase allospecifically. These data showed that the MHC molecule alone was a sufficient ligand for the interaction with alloreactive CTL.

Key words: Transplantation – Soluble HLA – Allo-antibodies – Inhibition – Alloreactive CTL – Serine esterase

Materials and methods

Patients. Venous blood was drawn from patients pre-transplant and at varying times thereafter, at which times biopsies were taken and examined histologically. Biopsies were staged according to Kemnitz et al. [1].

Quantitative Measurement of sHLA. sHLA were measured in a competitive ELISA assay as we have previously described [2].

Cytotoxic anti-HLA antibodies. Serum from patient RF was used in a cytotoxicity test using peripheral blood lymphocytes obtained from healthy blood donors. Sera were pre-diluted to 1:10 and 1:50 and used in a complement mediated cytotoxicity test. The test was performed on peripheral blood lymphocytes bearing the mismatched HLA-A3 and -B13. Sera were declared positive when they remained cytotoxic after pre-diluting to 1:10. The sera that remained negative were incubated with immuno-magnetic beads coupled with the anti-class I antibody W6/32. The beads were separated and the sera retested for cytotoxic antibodies. The anti-HLA antibody index was used such that 5 represented > 95% dead cells, 4 > 85%, 3 > 75%, 2 > 50% and 1 < 50%.

Generation of CTL. Alloreactive CTL were generated in vitro as we have previously described [3]. The stimulator and responder cells were selected such that CTL developed against HLA-A2.

CTL specificity and inhibition assays. The cytotoxicity of the alloreactive CTL was tested in a chromium release assay against PHA blasts, permanent tumor cell lines and EBV-transformed cell lines. To test the effect of sHLA, CTL were pre-incubated with varying concentrations of sHLA in 15% AB serum at 37°C for 30 min. Cells were harveted and washed 3 times in RPMI 1640 medium. Subsequently, cells were added to target cells which had been previously labelled with ^{51}chromium. The cells were incubated for 4 h before harvesting and measuring chromium release in a gamma counter.

Purification of papain-derived sHLA. Cell membranes were digested in papain, (Sigma, USA) at a concentration of 4 mg/ml as has been previously reported by Turner et al. [4]. The antibody PA2.1 (ATCC, USA), which binds HLA-A2 and -A28 was used to purify HLA-A2 polypeptides on affinity chromatography columns as we have previously reported [3]. The ME1 antibody (ATCC, USA) was used to purify HLA-B7 using the same procedure.

Serine esterase assay. The release of serine esterase was determined as has been previously reported by others [5].

Results

In our studies on the expression of sHLA in graft patients, we present a case report of a patient with vascular rejection. Patient RF maintained the cardiac graft for 29 days after which he rejected and died. During this period, there was no histological evidence of rejection on the biopsies examined. However, several peaks of sHLA elevation were noted as shown on Fig. 1. Retrospectively, we attempted to detect anti-allo antibodies. Three days post-transplant, cytotoxic antibodies against the mismatched HLA-A3 and -B13 could be identified. The antibodies gradually became less had completely disappeared by day 15. We depleted the negative sera of sHLA using immuno-magnetic beads to which the anti-class I antibody W6/32 was bound. These sera became positive for anti-

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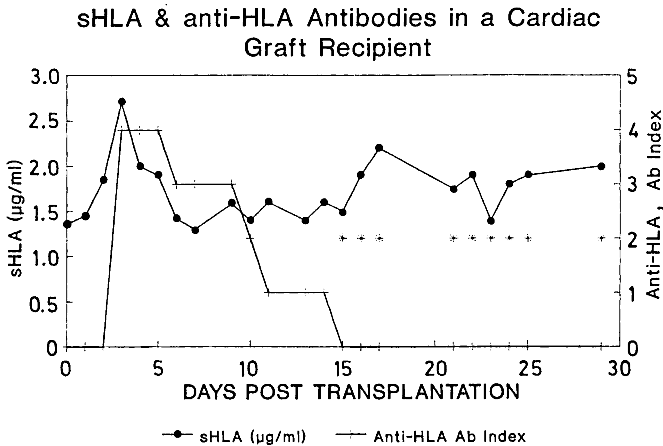

Fig. 1. sHLA (□) and cytotoxic anti-HLA antibodies (+) in a patient (RF) with vascular rejection. High increases of sHLA were noted soon after transplantation, between days 15 and 22 and between days 25 and 29. Anti-HLA antibodies were detectable only between days 3 and 15. Thereafter the sera were negative, but became positive (*) after sHLA depletion. The patient died of severe vascular rejection on day 29

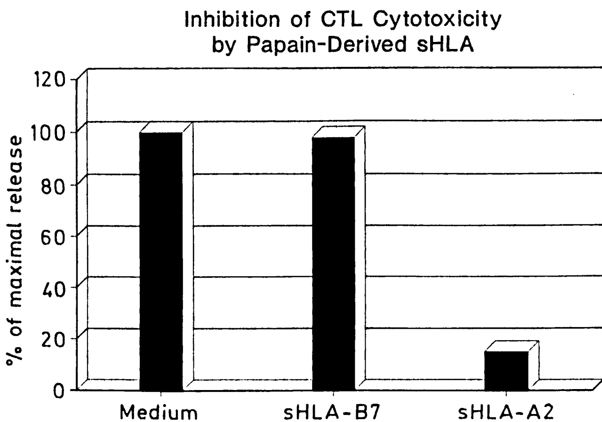

Fig. 2. Inhibition of CTL cytotoxicity by sHLA. CTL cytotoxicity was reduced to background levels by 1.25 µg/ml of papain-derived sHLA. Similar concentrations of sHLA-B7 were ineffective in abrogating CTL cytotoxicity

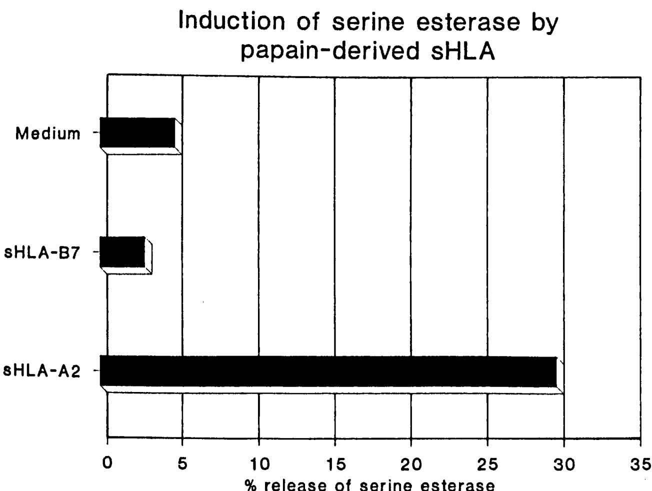

Fig. 3. sHLA triggered release of serine esterase by alloreactive CTL. The highest level of serine esterase was induced by the CTL by 0.15 µg/well HLA-A2 polypeptides. In the control experiment, HLA-B7 failed to trigger serine esterase release

HLA antibodies, as shown on Fig. 1, showing that antibodies had formed immune complexes with sHLA and could therefore not be detected in the cytotoxicity test. Post-mortem immunohistological examination of the graft showed deposition of complement factor C1q in the vascular bed (not shown). This finding was clear evidence of vascular rejection.

Patients who had acute rejection episodes expressed high sHLA levels at least 5 days preceeding histological evidence of rejection (not shown), confirming our previously published observations [6].

The CTL line generated was maintained in culture for several months. Only target cells that were HLA-A2 were lysed in the chromium-51 release assay. Over 98% of the cells were $CD8^+$.

In inhibition assays, CTL were pre-incubated with sHLA polypeptides before the chromium-51-release assays. CTL cytotoxicity was reduced to almost background levels by 1,25 µg/ml HLA-A2 (Fig. 2). Pre-incubation of the CTL with sHLA-B7 failed to influence CTL cytotoxicity. In a further attempt to investigate the molecular interaction of the CTL with the class I polypeptides, CTL were plated onto micro-titer plates which had been precoated with the polypeptides. The release of serine esterase into the supernatants was measured in an enzyme assay. Maximal release was measured at a concentration of 0.15 µg/well HLA-A2, whereas HLA-B7 polypeptides or culture medium failed to trigger the release of serine esterase (Fig. 3).

Discussion

In a previous report [6] we observed high elevations of sHLA during acute rejection episodes. In the present publication, we present a case report of a patient with vascular rejection who confirmed the earlier observations and showed that donor-derived sHLA form immune complexes with anti-HLA antibodies. Similar data have been reported by others [7, 8]. The formation of such complexes can potentially protect the graft from antibody-mediated tissue destruction. However, the possibility remains that they could increase the risks of graft rejection by sensitizing further the recipient. An important clinical point to consider is the fact that the presence of such complexes in pre-sensitized patients might lead to false negative crossmatches. Undetected antibodies in these patients could increase the chances of hyperacute rejection. Taken together, these data showed that sHLA measurement could be a useful indicator of rejection in graft patients and that they could play an important role in binding anti-allo antibodies. The inhibition studies suggested that sHLA are potential immunomodulators and could abrogate CTL cytotoxicity. The concentrations applied in these studies were reasonably low and allowed speculation on the in vivo clinical application of sHLA in inducing specific immunosuppression. Successful prolongation of graft survival using soluble class I molecules has been achieved in rats [9, 10]. These experiments demonstrated further that the interaction between sHLA and alloreactive CTL was allospecific, thus suggesting that the class I molecules reacted with the T cell receptor and either steri-

cally blocked it or triggered a negative signal preventing further interaction with target cells. Whatever the mechanism was, monomeric class I molecules were sufficient ligands for alloreactive T cells.

In contrast to the inhibition assays, immobilized papain-derived sHLA were effective in triggering CTL degranulation to release serine esterase, a CTL specific enzyme. Since this interaction was allospecific, it again suggested a direct interaction of the polymorphic regions of the class I molecule with the T cell receptor. Similar experiments have been reported in a murine model, where the influence of intact class I molecules was investigated [5].

In conclusion, the data presented here showed that sHLA are important molecules in allo-transplantation and that they are potentially effective immunomodulators on their own without other accessory molecules. We are furthering these studies to elucidate the mechanisms involved in their expression in vivo and their physiological functions.

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