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Transfected human class I gene product adequately assembles minor histocompatibility antigens

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The success of HLA genotypically identical bone marrow grafting is still hampered by graft vs host disease and rejection of the graft. One of the causes of the latter complication could be attributed to minor histocompatibility (miH) antigen disparities between HLA genotypically identical siblings (Goulmy 1988). In previous in vitro studies, we demonstrated the presence of anti-host cytotoxic T cell (CTL) as well as proliferative (Th) T cell reactions in blood-samples taken after HLA identical bone marrow transplantation (van Els 1990a, b). The latter activities are directed against miH antigens which are recognized in association with MHC class I (CTL) and class II (Th) products (Goulmy 1988; van Els 1990c). We recently acquired CTL clones specific for five non-sex-linked miH antigens designated HA-1, -2, -3, -4, and -5, whereby HA-1, -2, -4, and -5 use the HLA-A2 gene product as restriction molecule. Using the latter CTL clones, immunogenetic studies were carried out to determine the miH antigen gene frequencies and to study the miH antigen segregation patterns in families. One complication in these studies is that it is necessary for the HLA-A2 molecule to be present in order to detect the miH antigens. As illustrated in Figure 1a, the HLA-A2 positive identical siblings 02 and 03 carry miH antigens HA-1 and -2. Since the father (01) of family B only posseses HA-2, the miH antigen HA-1 must have been inherited from the mother. Therefore, the absence of the required MHC class I HLA-A2 restricting antigen hampers adequate genetic analysis in this and other families.

In order to solve this deficit, we used electroporation (Potter et al. 1984) to introduce cloned HLA genes into the Epstein-Barr transformed B cell lines of selected members of this family. We transfected the HLA-A2 gene cloned in the pHEBO vector (Sugden et al. 1985; Shimizu et al. 1986) into the HLA-A2 negative cells of the mother (00) and into three HLA identical, HLA-A2 negative family members 04, 05, and 06 (Fig. 1a). Fluorescence activated cell sorter (FACS) analyses using the class I and HLA-A2 specific monoclonal antibodies were carried out and demonstrated the surface expression of the HLA-A2 gene product on all transfected cells (Fig. 2). Subsequently, the transfected cell lines were subjected to miH typing. Figure 1b shows that we were indeed able to trace the miH antigen HA-1, which after *HLA-A2* gene transfection appeared to be inherited from the mother (00). Furthermore, the cells from three children (04, 05, 06) who did not carry the A2 allele and thus could not be tested for the presence of miH antigen HA-1 and -2, were now amenable to the CTL analysis. In addition to using the miH HA-1 and HA-2 specific CTL clones, we also tested HLA-A2 allo-specific and HLA-A2 restricted H-Y specific CTL clones on the cells of all family members. As shown in Figure 1b, A2 allo-specific, A2 restricted miH antigen H-Y, HA-1, and HA-2-specific CTLs lysed the transfected cells to the same extent as the naturally expressing HLA-A2 cells. To confirm that we were actually dealing with identical MHC/miH antigen peptide complexes, cold target inhibition studies were carried out. Table 1 shows that HLA-A2 transfectants were equally potent as cold target inhibitors of HLA-A2 directed lysis as their naturally expressing HLA-A2 counterparts. Non-transfected target cells failed to show any inhibition.

In conclusion, we have used gene transfection to circumvent the lack of the required MHC restriction molecule for miH antigens HA-1, -2, and H-Y recognition. Similar to the naturally expressed A2 gene products, the transfected gene products associate with the miH peptide(s) creating an assemblage suitable for A2/miH antigen specific T-cell receptor recognition. Recognition of human miH antigens upon implantation or by gene transfection of the required HLA antigens has been reported previously (Zier et al. 1987, Yamamoto et al. 1990). Our results are in line with both latter reports. The data shown here clearly demonstrate that gene transfection yields cell

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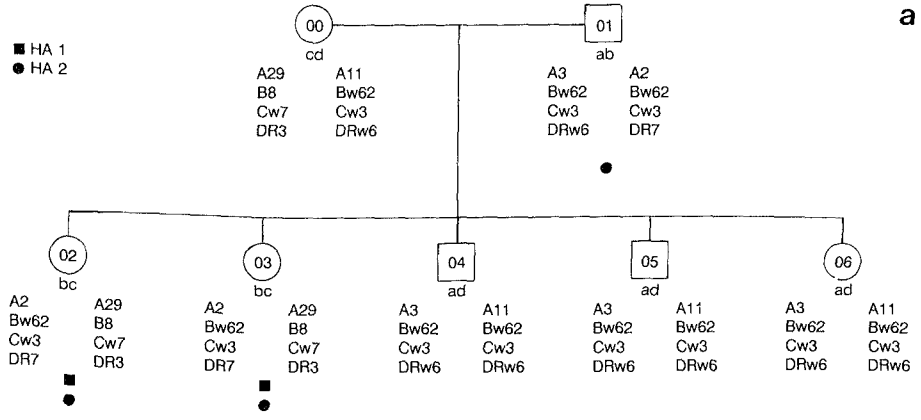

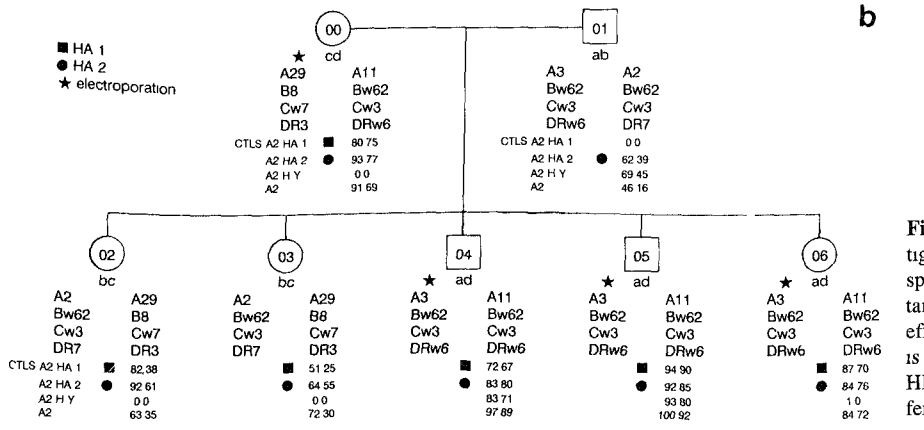

Fig. 1. a, b. The presence of the mιH antιgens HA-1 and HA-2 ιs ιndιcated by theιr specιfic symbols The % specιfic lysιs obtaιned from the dιfferent CTL clones at two effector to target ratιos (ι e , 20 1 and 2 1) ιs shown *ιndιcates that cells underwent HLA-A2 gene transfectιon, □ males, ○ females

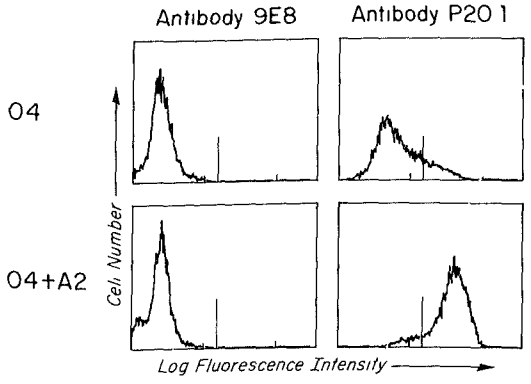

Fig. 2. The EBV LCLs of famιly members 00, 04, 05, and 06 were transfected by electroporatιon (Potter et al 1984) usιng the HLA-A2 gene cloned ιn the pHEBO vector (Shimιzu et al 1986) Selected cell growth was made possιble by the presence of hygromycιne B The cells were stained by a standard ιndιrect fluorescence technique usιng the ιndιcated monoclonal antιbodιes and FITC labeled goat antι-mouse and analyzed on a FACS analyzer

Table 1. Comparison of HLA-A2 transfectants and naturally HLA-A2 expressing cells as cold target inhibitors of HLA-A2 directed lysis

Target cells		Cytotoxic T cell clones			
hot	cold	allo A2	A2HY	A2HA 1	A2HA-2
00*	none	97/54†	1/ 4	90/81	70/25
00*	00	87/47		84/69	78/28
00*	00*	60/ 9		70/20	35/ 3
00*	04*	67/14		67/21	33/ 3
00*	03	74/28		87/56	44/ 6
04*	none	85/54	82/70	82/70	70/25
04*	04	87/65	82/64	89/64	66/25
04*	04*	49/12	65/17	55/22	26/ 3
04*	00*	52/18	84/74	57/16	36/ 7
04*	01‡	65/41	64/53	79/69	33/10
01	none	60/27	62/54	2/ 2	49/15
01	01	40/ 9	41/19		31/ 1
01	00*	16/ 0	67/50		21/ 3
01	04	57/33	71/47		56/15
01	04*	17/ 2	34/ 2		18/ 0
01	03	32/ 3	72/57		23/ 4
03	none	73/40	0/ 0	47/32	53/19
03	03	44/ 8		25/ 7	31/ 4
03	00	67/32		40/23	61/24
03	00*	30/ 3		7/ 0	30/ 7
03	04*	27/ 1		13/ 0	27/ 3

* Indicates the cells underwent transfection

† % Specific lysis at two effector/target ratio, i e , 20 1 and 2 1

‡ Note that 01 is HA-1 negative but HA-2 positive

The generation of mH antigen specific CTLs has been described in detail (Goulmy 1988) Specific cytotoxic T cell activity was measured by the cell-mediated lympholysis assay previously described (Goulmy 1982) The hot cold target cell ratio used is 1 10

surface expressed MHC products resulting not only in high levels of specific lysis by both allo- and MHC restricted mH antigen specific CTLs, but also in comparable inhibiting capacities. Consequently, gene transfection has proved to be a reliable technique for adequate mH antigen recognition and thus broadens its immunogenetic analyses

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