Regulation of CTL *in vivo*: Transition from Perforin- to FasL-based action

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Abstract Cytotoxic T Lymphocytes (CTL), along with natural killer (NK) cells, are key players in immune responses against certain intracellular parasites, viruses, in tumor and autoimmunity, and in the rejection of transplants. For a number of years now, our laboratory has been investigating the generation, mode of action of CTL at both the cell and molecular levels (reviewed in Berke and Clark 2005). The accepted hallmark of fully differentiated CTL is its perforin killing mechanism. Stored initially in secretory lytic granules, and co-secreted along with other granule constituents- such as granzymes- upon CTL interaction with its cognate target cell, it is believed that perforin and granzymes are the killing principles of CTL and of NK cells. An additional CTL killing mechanism is non-secretory, mediated by the cell surface death receptor Fas expressed on the target cell, interacting with FasL expressed on the CTL. Neither the rational nor the purpose for CTL possession of two distinct killing mechanisms has been proposed nor fully addressed. Using an *in vivo* mouse model of allograft rejection (fig. 1), we have found that CTL obtained from the rejection site early in the rejection process, are mainly fast-acting, perforin expressing cells, whereas those isolated later on are largely perforin deficient, slower killers killing their target primarily via the cell surface death receptor (fig. 2) Fas/FasL pathway (fig. 3). Based on our findings, we propose the following model for CTL development and action *in vivo*: at the beginning of an immune response the responding CTL populations are mainly perforin expressing CTL; as the immune response progresses the high perforin expressing CTL are eliminated, as a result of (antigen-) activation-induced cell death, combined with high Fas expression on the CTL, paving the way to the emergence of a largely perforin- and Fas- deficient CTL population, which are cytotoxic nevertheless but kill via FasL (fig. 4). Although late in emergence, these CTL are neither effector-memory, nor memory –CTL, as determined by cell surface phenotype and re-simulation analyses. These cells represent the continuous presence of CTL at the rejection site. Our findings suggest that as an immune response progresses, the CTL population switches its killing phenotype, from perforin to Fas/FasL based killing. This finding not only resolves previously contentious data concerning CTL biology, perforin expression and action, but also has serious clinical implications, as currently transplant rejection is monitored by assessing perforin expression in graft infiltrating lymphoid cells, which as shown here, is an early event and may go un-noticed at later stages of CTL responses in transplantation.

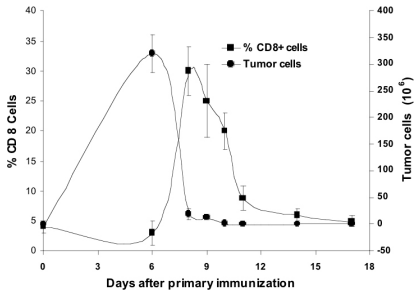

Fig. 1 *Kinetics of tumor growth and CD8⁺ T-cell response. Balb/c mice were injected i.p. with 25x10⁶ allogeneic EL4 tumor cells (H-2b). Peritoneal exudate cells were collected, counted and stained for CD8 and MHC haplotype (H-2b) to discern host CD8⁺ T cells and allograft EL4 tumor cells, respectively. FACS analyses provided the percentages of CD8⁺ versus EL4 (H-2b) tumor cells, excluding PI positive cells.*

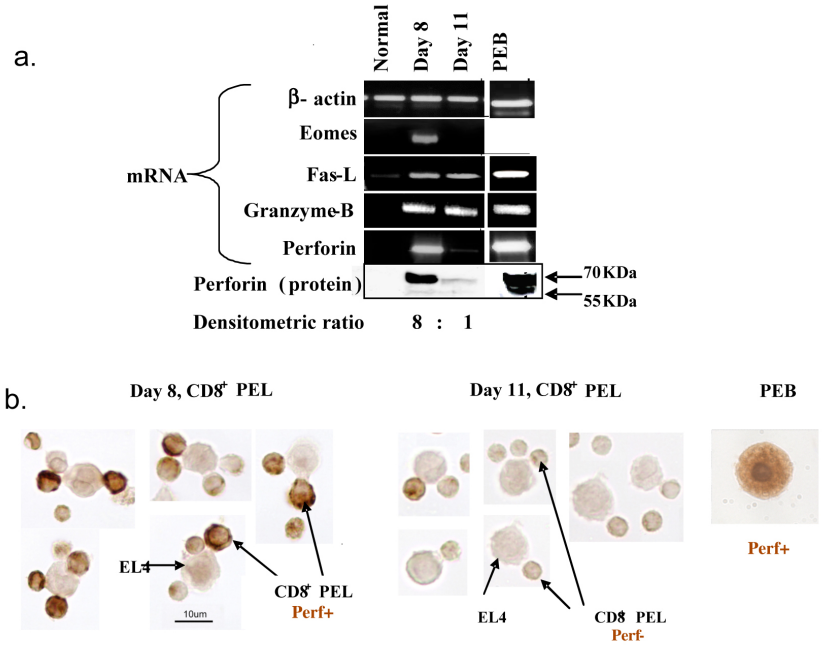

Fig. 2 *(a) mRNA expression of Eomes, FasL and Perforin in CD8⁺ T cells; (b) Perforin expression in CD8⁺ CTL.*

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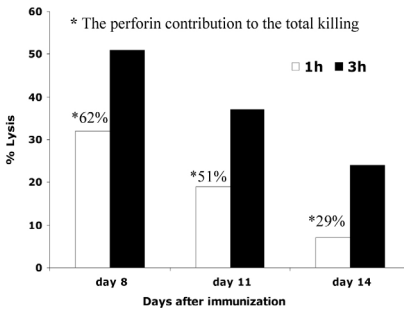

Fig. 3 *Perforin and Fas-based lytic activities.*
Balb/c anti-EL4 PEL-CTL were extracted 8, 11 and 14 days after tumor injection. The PEL-CTL were CD8⁺ FACS sorted and subjected to 1 and 3 hr lytic assay against 51Cr-labeled EL4. In the first hour of killing only perforin is employed; after three hours lysis was due to both Fas-FasL and perforin action.

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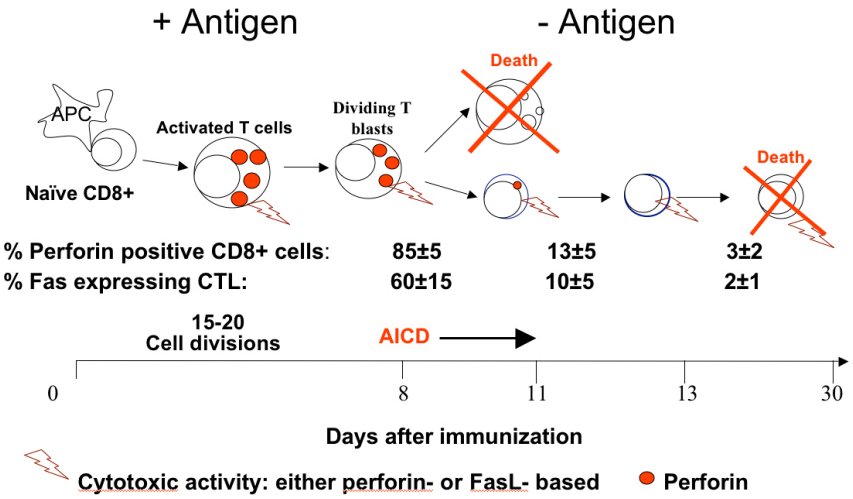

Fig. 4 *The transition from perforin to FasL -based CTL.*
Naïve CD8⁺ T cells activated by antigen-laden APC differentiate into dividing CTL blasts which continue to proliferate and differentiate to effector CTL. These CTL possess two main killing mechanisms, perforin/granzyme and FasL. As the immune response progresses and antigen is eliminated, a different kind of CTL, with different killing ability emerges. These CTL gradually cease to express perforin, relying mainly on their FasL activity, but are not memory CTL.

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