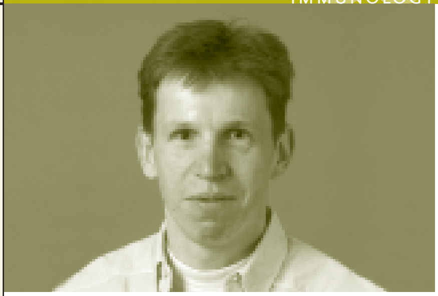
DISSECTING AND MANIPULATING ANTIGEN-SPECIFIC T CELL IMMUNITY

The aim of our research is straightforward 1) to design novel technologies that can be used to examine and modify antigen-specific T cell immunity 2) to use these tools to unravel and manipulate the molecular processes underlying immune recognition by T lymphocytes. Within these projects, a main focus is on the design and testing of novel concepts for adoptive immunotherapy.

Dissecting antigen-specific T cell immunity There is now substantial evidence that therapeutic manipulation of immune reactivity can result in clinically meaningful effects on human cancer. For example, T cell responses induced by either anti-CTLA4 treatment or infusion of ex vivo expanded tumor-infiltrating T cells (TIL therapy) have shown substantial activity in metastatic melanoma. Importantly, at present we do not know which cytotoxic T cell reactivities mediate cancer regression. Furthermore, as the number of potential melanoma-associated antigens to which these responses can be directed is very high, classical strategies to map cytotoxic T cell reactivity do not suffice. Knowledge of such reactivities would be of obvious use, both to monitor current therapies and to design more targeted strategies that selectively aim to induce immune reactivity against these antigens.
In the past years, we have set out to develop a broadly applicable platform that allows one to dissect disease- and therapy-induced T cell reactivity. First, in earlier work in collaboration with the group of Huib Ovaa (Division of Cell Biology) we have designed MHC class I molecules occupied with UV-sensitive 'conditional' peptide ligands, providing a robust platform for the creation of very large collections of pMHC complexes for T cell detection. Second, in more recent work we have developed a 'combinatorial coding' strategy that allows the parallel detection of dozens of different T cell populations within a single sample. Having established this technological framework, we have used it in collaboration with the Haanen group to assess whether TIL therapy induces a detectable alteration in the melanoma-reactive T cell repertoire. Using TIL cell products generated either at the NKI or by collaborators at NIH and in Israel, we have demonstrated that individual TIL products contain unique combinations of antigen reactivities, and that the combined magnitude of these responses is surprisingly low. Importantly, TIL therapy of melanoma patients leads to a significant increase in the tumor-reactive T cell compartment, and T cell reactivity post-infusion can almost in full be explained by the patterns of reactivity observed within the matched cell product. Collectively, these results suggest that the clinical efficacy of TIL therapy may be enhanced by the preparation of more defined tumor-reactive cell products, and establish the value of high-throughput monitoring for the analysis of immuno-active therapeutics.

Dissection of T cell immunity through genetic tagging and intravital imaging The ability to visualize antigen-specific T cell responses and to determine the differentiation pathways of different subsets of T cells is essential for our understanding of pathogen- and vaccine-induced immunity. While MHC tetramer technology makes it possible to follow the development of immunity at the T cell population level, it doesn't allow the analysis of cell fate and cellular differentiation pathways.
To allow for such lineage tracking, we have invested in the development of technologies with which individual T cells can be tagged with genetic barcodes. This tagging technology relies on the use of oncoretroviral and lentiviral libraries containing some 5,000 different barcodes. Infection of cell populations of interest by these libraries of viral vectors and subsequent analysis of the barcodes present within these cell populations can then be utilized to reveal lineage relationships. Using a strategy for the retroviral barcode labeling of naïve T lymphocytes, we have previously analyzed to what extent short-lived 'effector' T cells and memory T cells are derived from the same naïve T cell clones. Contrary to models that assume that T cell fate is to a large extent determined during the initial priming event, these data show that effector and memory T cell subsets are more or less completely derived from the same set of naïve T cell precursors. These data however did not address whether memory and short-lived effector fates could occur as a consequence



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Publications

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of an asymmetry during the first cell division, a model put forward by Reiner and colleagues. To address this possibility, we have now performed experiments in which genetic tags are introduced only after the first cell division. These data suggest that while the first cell division of T cells upon their activation may be asymmetric, the fate of the resulting daughter cells is purely symmetric.
The vast majority of studies – including those described above – primarily assess the development of antigen-specific T cell responses within the blood compartment. To also allow the analysis of T cell reactivity at the sites of action, the Haanen group and our group have developed an intravital imaging system in which virus-specific T cell responses can be followed in skin. Mathematical modeling of the data (collaboration with the group of Dr R de Boer, Utrecht University) has revealed by which migration pattern antigen-specific T cells within the periphery locate virus-infected cells. Furthermore, recent work on the imaging of memory T cells that remain in skin following clearance of infection indicates that the antigen-specific T cells that stay put take on a dendritic morphology and show a remarkable crawling behavior in between skin keratinocytes. The continuous migration of memory T cells allows these cells to contact large numbers of skin cells through time, and we propose that memory T cell crawling serves to allow the rapid detection of renewed infection at a previously infected site.

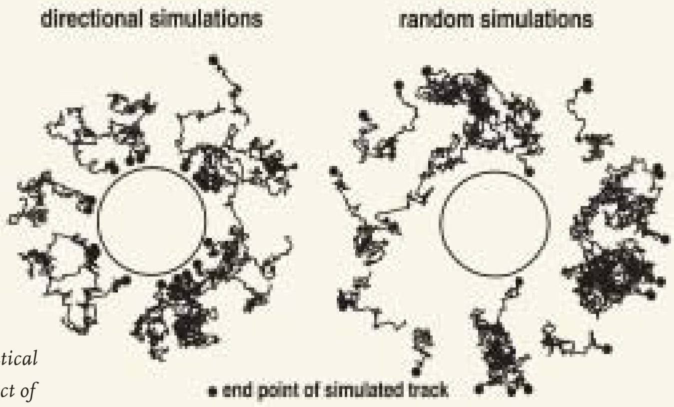

Figure 2: Mathematical modeling of the effect of directional migration of effector T cells on detection of virus-infected sites. Left: cell tracks of in silico cells that migrate using experimentally determined distributions of angles-to-infection. Right: tracks of in silico cells that migrate using randomly drawn angles-to-infection. Open circles indicate the simulated sites of infection. Closed circles indicate cell positions at the end of the simulation.

Adoptive T cell therapy (collaboration with Haanen lab) The cornerstone of our translational work is the development and evaluation of adoptive T cell therapies for human cancer. The MHC-based monitoring strategies described above will be utilized to evaluate T cell reactivity in the randomized trial for TIL therapy that will be carried out by the Haanen group. Furthermore, the material that is obtained in these analyses forms a very useful starting point for the further development of our second approach for adoptive T cell therapy; the genetic engineering of T cell reactivity. Specifically, in the past years our group has developed the retroviral introduction of antigen-specific T cell receptors into peripheral T cells as a means to induce tumor-specific T cell immunity. We have now produced a clinical grade batch retrovirus encoding a melanoma-reactive TCR for the gene modification of T cells of patients with metastatic melanoma. The pharmaceutical process that will be utilized to generate gene-modified T cells for this first NKI TCR gene therapy trial has been established. To evaluate which antigens can best be targeted in TCR gene therapy, we are at the same time preparing for a substantially larger clinical program in which we intend to test a series of different T cell receptors that target various tumor-associated antigens. The TCRs that we aim to use in this program will be derived from the antigen-reactive T cell populations identified in our TIL monitoring program, and we are currently establishing novel technologies to retrieve such TCR genes. Furthermore, we are evaluating alternative, transposon-based gene transfer strategies to create the TCR-modified T cells that can be used in these studies.