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Imaging T cells *in vivo*

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There is a growing pharmaceutical and biomedical interest in the opportunity to boost or inhibit the activity of the immune system at the site of disease. The experimental demonstration that most human tumors contain at least one mutation per megabase [i.e., more than 1000 mutations in the whole genome; 1] suggests that various mutated peptides may be presented on MHC class I molecules (HLA class I in man) and be recognized by specific T cells, thus serving as potential "tumor rejection antigens". The existence of such peptides provides a strong rationale for the design and implementation of immunotherapeutic strategies against various types of malignancies. More broadly, T cell-based recognition of peptides presented on MHC class I and class II molecules is crucially important for a broad variety of disease conditions, including chronic inflammatory processes, organ rejection, autoimmunity and allergy.

Antibodies which target the inhibitory proteins CTLA-4 and PD-1 act as potent stimulators of the immune system and have gained marketing authorization (or are in advanced clinical trials) for the treatment of various types of cancer, including metastatic melanoma [2-4]. Moreover, antibody-cytokine fusion proteins, capable of selective localization in cancer or at sites of chronic inflammation, are currently being investigated in clinical trials, with the potential to selectively boost or inhibit immunity at the site of disease [5-9]. These and other immunomodulatory strategies crucially impact on the activity of T cells. In addition, the genetic engineering of artificial receptors on T cells is opening new avenues for the treatment of hematological malignancies and, potentially, other tumor types [10].

The opportunity to study T cell activities in humans are often limited and, in most cases, restricted to the *ex vivo* analysis of lymphocytes in biopsies (e.g., staining of lymphocyte infiltrates) or in blood (e.g., by the tetramer-assisted investigation of T cell specificities by FACS [11,12]). The ability to image T cells *in vivo* could open novel biomedical opportunities, both for the study of fundamental immune processes in health and in disease, as well as for the monitoring of responses to pharmacological intervention.

In this issue, Anna Wu and collaborators describe the successful imaging of secondary lymphoid organs, rich in T cells, using radiolabeled monoclonal antibodies, directed against the CD4 or CD8 T cell antigens [13]. Specifically, the authors used antibodies in diabody format, which is cleared more rapidly than the corresponding parental IgG format and is thus more suitable for *in vivo* imaging applications [14,15]. The radiolabeled antibody fragments, which were specific to mouse CD4 and CD8 antigens, nicely imaged lymphnodes and spleen in immunocompetent mice, with excellent selectivity (as confirmed by the quantitative assessment of percent injected dose per gram of tissues in various organs and body structures). A number of control experiments, including the administration of radiolabeled antibodies after a suitable lymphocyte depletion step with unlabeled antibodies, were performed in order to confirm the specificity of the imaging procedure.

The article is, in my opinion, important for a number of reasons. First of all, the authors nicely show that the CD4- and CD8-specific diabodies are not trapped in blood by circulating lymphocytes, which are present at a lower density, compared to solid structures in secondary lymphoid organs. Secondly, the experiments suggest that it may be possible to gain information about T cell density in certain tissues (e.g., in tumors) from the corresponding signals in Nuclear Medicine investigations.

What can we expect from future development in the field? On one hand, it will be important to experimentally validate whether T cell infiltrates in tumors can be quantitatively assessed in rodent models of cancer and in patients, using suitable diabody-based imaging agents. Furthermore, similar imaging strategies could potentially be used for the *in vivo* monitoring of engineered T cell specificities [e.g., those clinically used in Chimeric Antibody Receptor (CAR) technology; 10]. More broadly, the availability of surface markers for defined subsets of T cells (or other leukocytes) may allow a "finer" quantification of cellular infiltrates in organs and diseased tissue. Molecular imaging techniques may therefore become extremely important for the industrial and clinical development of immunomodulatory agents.

What will be the main future challenges? First of all, distinctive surface markers for many "important" T cell subtypes (e.g., regulatory T cells) are not yet available. Secondly, signal-to-noise in imaging procedures may decrease, if antibodies that target rare lymphocyte populations are used. On the other hand, the extensive knowledge available for the expression patterns of Cluster of Differentiation ("CD") antigens on leukocytes in health and disease would deserve imaging investigations, with affinity reagents similar to the ones described in the current study [13]. In this respect, CD4 and CD8 may only represent the beginning of a more extensive series of Nuclear Medicine investigations. Finding a balance between the regulatory need to comply with "Good Manufacture Practice" (GMP) guidelines and the need to clinically investigate various types of antibody molecules will also represent a formidable challenge in Translational Medicine.

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