Imaging cellular communication and motility in the live immune system

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A hallmark of leukocytes is their ability to migrate among tissues. This allows them to communicate with changing cellular partners throughout development, notify cells in the lymph nodes and spleen about infections in distant organs of and co-ordinate immune responses. In recent years, multiphoton microscopy has revolutionized our ability to track cells in live animals and gain new insights on immune function. We establish a new laboratory in the Department of Immunology to study a diverse set of processes in the immune system. All of these processes have three features in common: they are highly dynamic, they depend on multicellular communication and they can only be studied in intact animals. Major focuses of the lab are **T cells** and **dendritic cells** (DCs).

For that aim we are operating a multiphoton imaging system consisting of a powerful pulsed Ti-Sapphire laser, which is tunable and powerful as well as a multiphoton microscope equipped with highly sensitive light detectors and optimized optics to maximize light collection. The multiphoton imaging system is central to the operation of the lab and is supplemented by classical immunological methodology, *in vitro* microscopy and histology. Students in the lab study the following study using the system:

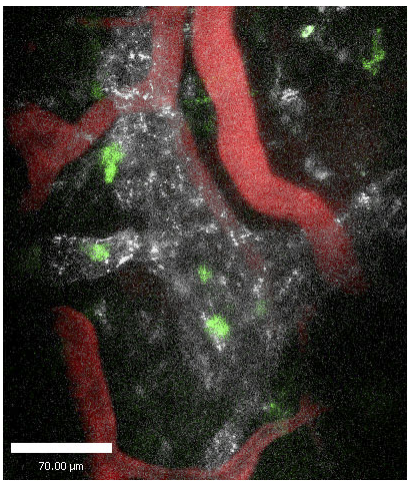

Fig. 1 *Live two-photon imaging of an inflamed footpad of a mouse, shows CD11c-EYFP dendritic cells (green), which have migrated into lymphatic vessels (white) marked by LYVE-1 antibody. Blood vessels are seen in red (Qdots 655).*

Requirements for dendritic cell migration from the skin to the lymph nodes

For the immune system to gain knowledge of pathogens invading the skin, and for skin-based vaccination to work, a specialized cell, called a dendritic cell (DC) needs to migrate from the skin to lymph nodes carrying antigens it sampled from skin pathogens (antigen). The molecular and morphological changes that enable DCs to enter lymphatic vessels and reach lymph nodes are poorly defined, as they cannot be studied in a dish. We are using multiphoton microscopy to determine whether, upon encountering bacteria in the skin, DCs stop migrating and enter small lymphatics. We study the anatomic route DCs use to enter lymphatics and dissect the molecular signals involved in their interaction with the cells lining the lymphatic vessels. This is done by imaging the skin of genetically-engineered mice whose DCs endogenously express eYFP, while pharmacologically and genetically interfering with various molecules that participate in DC migration. We devised a way to follow DC migration in intact skin, and began to look at DC interaction with the lymphatic vessels that DCs use to reach lymph node. We have already shown that normally immobile DCs in the skin start to patrol this tissue in search of bacteria as soon as they sense a bacterial invasion. We can now follow DCs as they become activated and interact with cells of the lymphatic vessels. Upon inflammation we observe dermal dendritic cells crawling inside the lymphatics (Fig. 1). We are currently examining if the various stages in DC migration are impaired in mice deficient in the molecules CCR7 and the integrin LFA-1, known to be important for DC arrival into the lymph node.

Encounters of T cells and DCs guided by transfer of antigen through gap junctions connecting DCs

For a successful immune response to a skin pathogen or cutaneous vaccination, two rare cells – a DC carrying antigen and a clonotypic T cell expressing a T cell receptor specific for the antigenic epitope – must find each other in the lymph node. We previously showed that DCs form stable networks in the T zone and that newly immigrating DCs, injected into the skin, join these existing networks. Studies show that T cells migrate vigorously on these networks before they locate the right DCs to interact with – the one carrying the optimal amounts of antigen. Other studies show that DCs use gap junctions – specialized pores that connect adjacent cells – to transfer antigen among themselves. We hypothesize that antigen is transferred among DCs in the lymph node through gap junctions, establishes gradients of antigen presented on the surface of DC networks and allowing T cells to locate the DCs carrying most antigen. We use multiphoton microscopy to visualize this process in live mice. We have now devised ways to reconstitute DC networks *in vitro*, track T cell motility on *in vitro* networks and in lymph

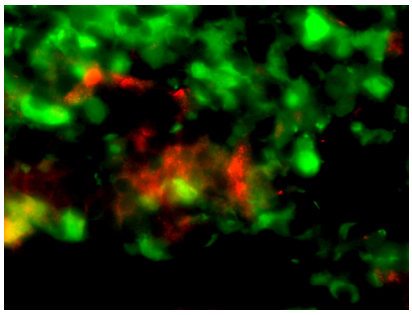

Fig. 2 *Dendritic cells in an inflamed lymph node (green) express connexin43 – a gap junction protein (red). This indicates that transfer of peptides through gap junctions among dendritic cells in the lymph node is feasible.*

nodes and we are attempting to follow fluorescent antigens as they moves within the network of DCs. Our initial results indicate that DCs express gap junction molecules both in vitro and in lymph nodes (Fig. 2).

Mechanisms of tumor cell resistance to T cell killing

Melanoma patients can mount an immune response against the tumor, as evidences by T cells that can selectively kill tumor cells which proliferate and infiltrate the tumors. Although these cytotoxic T cells (CTLs) can kill melanoma cells in the dish, they usually fail to stop tumor development – partially because the tumor cells induce an immunosuppressive environment inside the tumor. We are trying to observe the interactions between CTLS and tumor cells within B16 melanomas, comparing successful and abortive anti-tumor responses to pinpoint the phases in which the CTLs are vulnerable to immunosuppression. For this aim we are developing a method to microscopically follow the interactions between CTLs and melanoma cells inside intradermal tumors in mice. We intend to follow the events that lead to tumor cell killing, determining how efficiently T cells locate the tumor cells, adhere to them, and release toxic molecules to eliminate them. The immunosuppressive molecule we are focusing on is TGF-beta. A major tool for deciphering its role is the dnTGF-R mouse, whose T cells do not respond to TGF-beta and are less prone to intratumoral immunosuppression. So far we have developed new fluorescent tumor cell lines for use as target cells and have devised a method to image tumor cell killing by T cells in live mice.

The molecular anatomy of bone marrow cell mobilization

In response to injury, bleeding and stress, white blood cells leave the bone marrow into the blood. Mobilization, as this process is called, can also be induced clinically to collect hematopoietic stems cells for bone marrow transplantation. Although there has been progress in identifying the molecules that participate in bone marrow mobilization, the cellular interactions and dynamics of the response are still poorly understood. We are transplanting green-fluorescently tagged bone marrow cells into mice whose endothelium is tagged with GFP. The interactions of these 2 cell types are recorded in quiescent and mobilized conditions and genetically manipulated mice are used to pinpoint the role of various proteins in this process. We have perfected a method for imaging fluorescent bone marrow cells in the skulls of anesthetized mice. Many cells exhibit vigorous motility in the bone marrow cavity, while some maintain stable contact with osteoblasts. Upon mobilization with the CXCR4 agonist AMD3100, we demonstrate that white blood cells accumulate near the blood vessel endothelium.

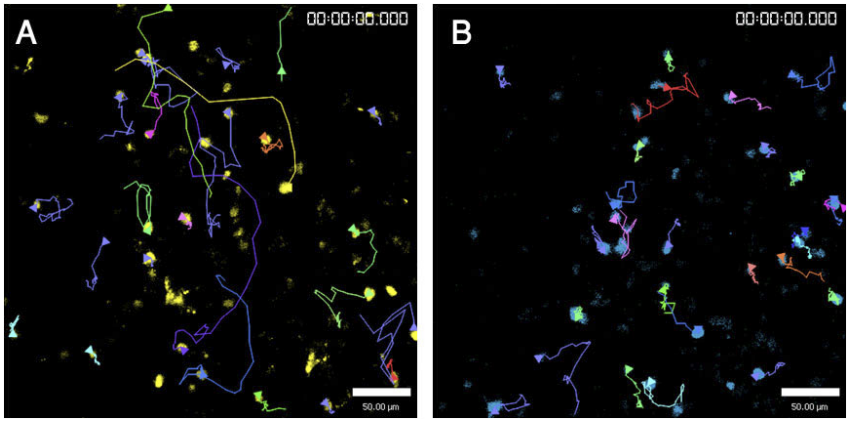

Fig. 3 *The motility of adoptively transferred mature T (A) and B cells (B) in the bone marrow of an anesthetized mouse. Tracking of the cells over 30 minutes shows that T cells move faster and exhibit less confined trajectories than B cells.*

Immunological niche in the bone marrow

The labs of Steffen Jung and Idit Shachar recently reported that DCs in the bone marrow define a novel niche dedicated for hosting mature B and T cells and supporting mature B cell survival. In collaboration with these labs, we are examining whether this structure, coined the immunological bone-marrow niche, can also support immune responses, studying the motility and activation of mature cells participating in immune responses within these niches. Our initial examination identified the baseline behavior of these cells in the bone marrow. While B cells were confines to small areas in the niches, adjacent to $CD11c^{+}$ DCs, T cells moved more freely, traversing the BM cavities (Fig. 3).

Control of inflammatory bowel disease by regulatory T cells

In collaboration with the lab of Prof. Zelig Eshhar, we are initiating a project to study the amelioration of inflammatory bowel disease by regulatory T cells (Tregs). We can visualize regulatory T cells, marked with GFP driven by Foxp3 and we can externalize the colon to watch the behavior of leukocytes in the lamina propria of live mice. We intend to study the tripartite interaction of pathogenic effector CD4 T cells, Tregs and DCs to better understand the mechanism used by Tregs to control inflammation.

Selected publications

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