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# Tracking and treating activated T cells

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## Abstract

Upon activation, T cells of various subsets are the most important mediators in cell-mediated immune responses. Activated T cells play an important role in immune system related diseases such as chronic inflammatory diseases, viral infections, autoimmune disease, transplant rejection, Crohn disease, diabetes, and many more. Therefore, efforts have been made to both visualize and treat activated T cells specifically. This review summarizes imaging approaches and selective therapeutics for activated T cells and gives an outlook on how tracking and treating can be combined into theragnositc agents for activated T cells.

### Keywords

Activated T cells; Imaging; Treatment; Theragnostics; siRNA; Targeting

T cells are lymphocytes that mature in the thymus and can be identified by their expression of the T cell receptor (TCR). Based on their maturation process, T lymphocytes can differentiate into several different subsets, each of which has different effector functions and molecular phenotypes [1]. Examples of subsets are $CD4^+$ T helper cells that can be further differentiated into $T_H1$ cells which mediate cellular immune responses, $T_H2$ cells responsible for humoral immune responses, $T_H17$ cells which play a key role in autoimmune diseases and anti-microbial immunity, $T_{FH}$ cells located in the follicular regions of secondary lymphoid organs, and $T_H3$ cells producing TGFβ [2, 3]. Another subset are the cytotoxic $CD8^+$ cells (CTL cells) that clear tumor cells and cells infected by viruses [4]. Regulatory T cells ($T_{Reg}$ cells) suppress the immune response, and natural killer T cells (NKT cells) are cytotoxic for a variety of target cells [2].

T cells are one of the major components in cell-mediated immune responses. The interaction of T cells with antigen-presenting cells (APCs), through the T cell receptor recognition of peptides presented by the major histocompatibility complex and the costimulation by CD28 immunoglobulin superfamily members on T cells binding to B7 family members, initiates a series of signaling cascades resulting in T cell activation [5, 6]. B7 family members on APCs are upregulated after activation and the B7:CD28 costimulatory signal augments the TCR signal, and thereby promotes T cell response [7]. The activation of T cells is triggered either by antigen-presenting cells or other target cells. The activation process itself is complicated and involves the cytoskeleton, as well as integrin-mediated adhesion, receptor sequestration and other intracellular steps [8].

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Activated T cells play a key role in immune response and immune system related diseases such as chronic inflammatory diseases, viral infections, autoimmune disease, transplant rejection, Crohn disease, diabetes, and many more [9-12]. Therefore, the selective detection and tracking of activated T cells are very useful for the diagnosis of many inflammatory or infectious diseases as well as for the follow-up of treatment effects. Furthermore, a specific treatment targeting activated T cells bears a therapeutic benefit since the activated rather than naïve T cells play a central role in immune response cascades.

Keeping the importance of activated T cells in mind, in this review, we focus on the selective imaging and treating strategies of only activated T cells, distinguished from naïve T cells or other immune cells.

# I. IMAGING ACTIVATED T CELLS

While non-invasiveness, cost-effectiveness and sensitivity are absolutely important, selective detection of activated T cells and differentiation from naïve T cells are the key aims in diagnostic imaging techniques. Therefore, for most studies involving the tracking of activated T cells specific probes were developed to detect markers that are specifically expressed on the cell surface of T cells upon activation.

Once the T cell receptor recognizes a peptide antigen presented by the major histocompatability complex on the antigen-presenting cell, a series of signaling cascades are initiated and result in expression and secretion of several cytokines and up-regulation of their receptors, which can be a good marker for T cell activation [13]. Interleukin-2 (IL-2), a small single-chain glycoprotein expressed and secreted by activated T cells, has been widely used to detect activated T cells [14]. Becker *et al.* utilized transgenic mice (IL-2/GFP$^{ki}$) that co-express the green fluorescence protein (GFP) gene together with IL-2 after insertion of cDNA coding for GFP into the locus of IL-2 [15]. Activated T cells infiltrating the iris stroma in mice with endotoxin-induced uveitis could thus be visualized by enhanced GFP fluorescence induced by the up-regulation of IL-2 using intravital epifluorescence miscoscopy at multiple time points. Using the specific binding affinity of IL-2 to the IL-2 receptor expressed on activated T cells, radioisotope $^{123}$I-labeled IL-2 has also been investigated for *in vivo* imaging of activated T cells. In a mouse model for the study of the pathogenesis of type 1 insulin-dependent diabetes (IDDM), high accumulation of $^{123}$I-IL-2 was observed in the pancreatic region, suggesting the possibility to use nuclear imaging for the early diagnosis of IDDM [16]. Intravenously injected $^{123}$I-IL-2 to renal allograft transplanted rats showed the selective and enhanced retention of the radioactivity compared to non-rejecting grafts measured by non-invasive gamma camera imaging [17]. In addition to $^{123}$I, $^{99m}$Tc labeled IL-2 was also studied for the radio-labeling process with a single-step synthesis method to improve cost and time factors [18]. However, *in vivo* activated T cell imaging with $^{99m}$Tc-IL-2 has not been reported yet. Positron emission tomography (PET), which is more sensitive and offers higher resolution, was also reported for the visualization of IL-2 receptor positive T cells [12]. Activated T cells which were subcutaneously injected into the shoulder of immune-depressed SCID mice were clearly visualized after intravenous injection of N-(4-$^{18}$F-fluorobenzoyl) IL-2 by PET imaging. In addition to the IL-2 receptor, interleukin-12 (IL-12) receptor is also a specific target for the detection of activated T cells, since IL-12 receptor is up-regulated upon activation of T cells or NK cells [19]. Radiolabeled $^{99m}$Tc-IL-12 has shown specific accumulation in inflamed areas of the colon in 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced chronic colitis mice, while accumulation in noninflamed areas or control mice was not significant [20].

Other than cytokines, T cell receptor-dependent nuclear factor of activated T cells (NFAT), which is a major transcription factor downstream of the T cell receptor/CD3 signal cascade,

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has been used for the tracking of activated T cells with NFAT-inducible reporter systems [21-23]. Na *et al.* recently reported a dual bioluminescent reporter system consisting of a constitutive reporter and NFAT-activation inducible reporter that can non-invasively monitor *in vivo* trafficking of activated T cells in a mouse model of graft-versus-host disease (GVHD) [22]. Ponomarev *et al.* developed the herpes simplex virus type 1 thymidine kinase/GFP protein (TKGFP) dual reporter gene for imaging of NFAT-mediated activated T cells [21]. Using the same reporter system, they demonstrated optical fluorescence imaging as well as PET imaging with $^{124}$I-FIAU (2'-fluoro-2'deoxy-1-β-D-arabinofuranosyl-5-iodouracil) to visualize activated subcutaneous Jurkat infitrates transduced with NFAT-TKGFP reporter in nude mice [21].

Furthermore, a therapeutic agent that has a selective toxicity toward T cell lymphoblasts, 9-(β-D-arabinofuranosyl)guanine (AraG) was tested as a PET imaging probe after radiofluorination [24]. Although *in vitro* or *in vivo* results of PET imaging with [$^{18}$F]F-AraG have not been reported, higher uptake of [$^{18}$F]F-AraG into activated primary T cells suggests a potential to be used as a PET imaging probe in the diagnosis of diseases that involve activated T cells. PET probes like 1-(2'-deoxy-2' [18F]fluroarabinofuranosyl)cytosine ([$^{18}$F]FAC ) that use the deoxyribonucleotide salvage pathway, which is mostly utilized in lymphoid organs and rapidly proliferating tissues, have shown a higher accumulation in activated T cells ex vivo and an increased lymphoid mass in autoimmune disease model mice compared to wild-type mice [25]. However, the specificity for activated immune cells should be further studied *in vivo* before these probes can be applied as a specific PET probe for activated T cells.

In addition to the imaging strategies using activated T cell-specific probes described so far, histological analysis, one of the classical imaging techniques, has been employed to confirm the probe-mediated imaging results in some studies [16, 20]. Two-photon laser scanning microscopy (TPLSM) has shown the different migration pattern of activated and naïve $CD4^+$ T cells in autoimmune CNS inflammation models [26, 27]. Magnetic resonance imaging (MRI) is another noninvasive and highly sensitive imaging technique, but *in vivo* visualizing of activated T cells has not been reported yet to our knowledge. An *in vitro* study demonstrated the feasibility of imaging activated T cells isolated from rhesus macaques showing successful labeling with monocrystalline iron oxide nanoparticles (MION) [28].

## II. TREATMENT OF ACTIVATED T CELLS

Since activated T cells are involved in various inflammatory diseases such as asthma, autoimmune diseases, and acute rejection after organ transplantation, treatment strategies to target activated T cells have been developed using markers on the cell surface of activated T cells. One such approach that is extensively employed and showed successful results is antibody-based. In this approach, the cell surface markers on the activated T cell are blocked by an antibody against a specific marker resulting in inhibition of the signaling pathway or induction of active cell death.

One of the chronic inflammatory diseases in which activated T cells play a central role is asthma, which is an inflammatory disorder characterized by chronic airway inflammation caused by infiltration of eosinophils and $T_H2$ cells in the lung [29]. In the airways of asthmatics, many of the inflammatory cascades are orchestrated by $CD4^+$ T cells, which secrete IL-4, IL-5, and IL-13 [30], as shown in *Figure 1*. It was shown that in asthma-mediated lung inflammation, inflammatory responses caused by activated $CD4^+$ T cells were decreased by using an anti-CD147 antibody that modulated the activated cell surface signaling [31]. Equivalently, a monoclonal anti-E-selectin (agonist) antibody against the cutaneous lymphocyte antigen expressed on activated T cells in atopic skin inflammation

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helps to reduce inflammatory reactions [32]. In graft versus host type autoimmune disease, upregulation of CD44 expression causes inflammation and rejection. An anti-CD44 monoclonal antibody (mAb) that killed activated T cell was shown to prevent the graft rejection and associated negative responses [33]. And in acute lymphoblastic leukemia, targeting or blocking the mammalian target of rapamycin (mTOR) on activated T cell was reported to inhibit primary leukemia [34, 35]. Bispecific antibodies can also be used in targeting activated T cell and other cell surfaces or two different surface markers on the same activated T cell. A bispecific antibody that was chemically crosslinked and genetically engineered with specificities for tumor and CD3 on activated mouse T cells successfully killed tumor cells in primary breast cancer [36]. Activated T cell targeting was extensively studied and tested clinically in rheumatoid arthritis. Several of these strategies included the cytotoxic T lymphocyte antigen 4 (CTLA4, CD152) on activated cytotoxic T cells, the CD4 surface marker on T helper cells, the T cell receptor on activated T cells in disease state synovial fluid, and co-stimulatory signal molecule blockade of CD28/B7 [37-41].

Activated T cells are also treated by using indirect methods such as modulating cytokines, tumor necrosis factors, peptides, or co-stimulatory molecules [42]. In inflammatory bowel disease, antibodies against TNF caused apoptosis of activated T cells and thus reduced the inflammatory symptoms [43]. Synovial homing peptides that targeted the synovial vasculature have decreased the inflammation in autoimmune rheumatoid arthritis by decreasing T cell trafficking to the synovial region [44]. In cardiac allograft rejection, T cell to T cell mediated co-stimulation in activated T cells was targeted and blocked with a co-stimulatory signal/ligand [45]. Activated $CD4^+$ T cells expressing tumor necrosis factors were also blocked by ligand-mediated interaction (such as OX40 or CD134) and thus minimized autoimmunity [46]. In situations where T cells are activated, combined strategies that employed an antibody conjugated to chemotherapeutic or photodynamic agents that target and kill activated T cells were also shown to be an attractive strategy [47]. Activated T cells showed higher sensitivity to photodynamic treatment with benzoporphyrin derivative monoacid ring A compared to resting T cells [48]. A synthetic peptide analog that targeted T cells and blocked the high voltage-gated potassium channel (Kv1.3) helped in reducing a delayed type of hypersensitive reactions [49-51].

Small compound drugs or natural products have also been tested for their effect on activated T cells. Tacrolimus, an immunosuppressive drug inhibiting activated T cells specifically, reduced the number of activated T cells in cerebrospinal fluid (CSF) and activated cerebellar degeneration-related protein 2 (cdr2)-specific cytotoxic T lymphocytes in the peripheral blood of patients with paraneoplastic cerebellar degeneration [52]. The effects of bromelain, an extract from pineapple stem acting as an anti-inflammatory agent, on activated T cells have been various. Decreased number of activated $CD4^+$ T cells in an allergic airway model and reduced expression of CD25, which is upregulated upon T cell activation, on anti-CD3 stimulated $CD4^+$ T cells were demonstrated, while Hale *et al.* showed bromelain treatment enhanced CD2-mediated T cell activation [53-55]. These results may be due to the variability in the immunomodulatory properties of natural products [56, 57]. Other natural compounds such as astin C, a plant cyclopeptide isolated from Aster tataricus roots, or fraxinellone, a natural small lactone isolated from Dictamnus dasycarpus root bark, induced apoptosis of activated T cells *in vivo* in murine colitis or hepatitis models [58, 59].

Gene therapy can be another powerful method to treat activated T cells, however, studies about gene-mediated treatment of activated T cells in the literature are very limited because of the inefficient delivery to primary T cells *in vitro* and *in vivo*. Various delivery strategies such as electroporation, nucleofection, viral or non-viral vectors, have been studied to transfect T cells [60-63]. Even though some of the studies reported high transfection efficiency in activated primary T cells, specific delivery of DNA or siRNA to activated T

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cells was not demonstrated. One strategy for targeted delivery to activated T cells is to use transferrin as a targeting ligand for non-viral vectors. Activated T cells highly express the transferrin receptor (TfR), which is internalized into the cell by receptor-mediated endocytosis after binding with transferrin [64]. Using this characteristic, our group has examined the siRNA delivery efficiency with a transferrin-low molecular weight polyethylenimine conjugate (Tf-PEI) into activated primary human T cells [65]. We found the specific uptake of fluorescently-labeled siRNA complexed with Tf-PEI into TfR-overexpressing T cells compared to unconjugated PEI (*Figure 2a*), while the uptake level was similarly low for both Tf-PEI/siRNA and PEI/siRNA in T cells with low TfR expression level (*Figure 2b*). Based on this result, we expect that radiolabeled siRNA delivered with Tf-PEI can be used for both therapy and diagnosis of activated T cell related diseases, such as asthma.

While activated T cells have so far only been specifically tracked or treated, a combined theragnostic agent has not been described yet. Most approaches to image activated T cells specifically used probes for IL-2 which were not employed further for therapeutic effects. The most efficient selective therapeutics for activated T cells that are described in the literature are antibody-based. These therapeutics could essentially be radiolabeled and used as theragnostics, for example to image T cell trafficking, recruiting to sites of inflammation or to lymph nodes. To enhance the therapeutic effects, interference with disease-causing transcription factors like GATA-3 in $T_H2$ cells, rather than downregulating single cytokines, has been reported to be a promising approach [66] and will certainly gain importance in the future. Therefore, RNA interference technology will certainly gain importance and can serve as an attractive theragnostic approach in the future.

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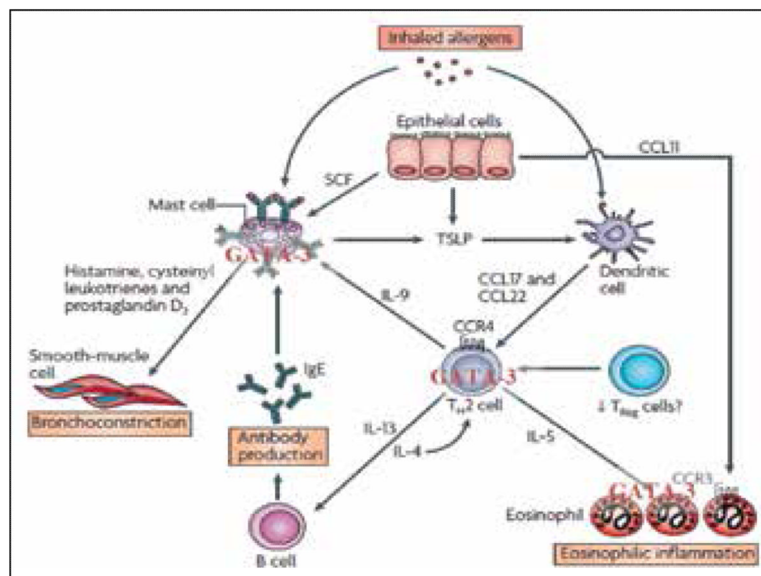


**Figure 1.**

Pathophysiology of asthma involving activated T cells. Adapted from Barnes, et al [67]. T cell activation by antigen-presenting cells (APCs) induces translocation of GATA-3 into the nucleus and thus upregulates gene expression of $T_H2$ cytokines, which leads to i) increased mucus production in goblet cells, ii) activation of eosinophils and B cells. The latter produce IgE, which is presented on the surface of mast cells to directly interact with the allergen. Both eosinophils and mast cells trigger smooth muscle broncho-obstruction mediated by secretion of second messengers such as leukotriens, cytokines, and histamine. Thus, GATA-3 conducts an orchestra of inflammation.



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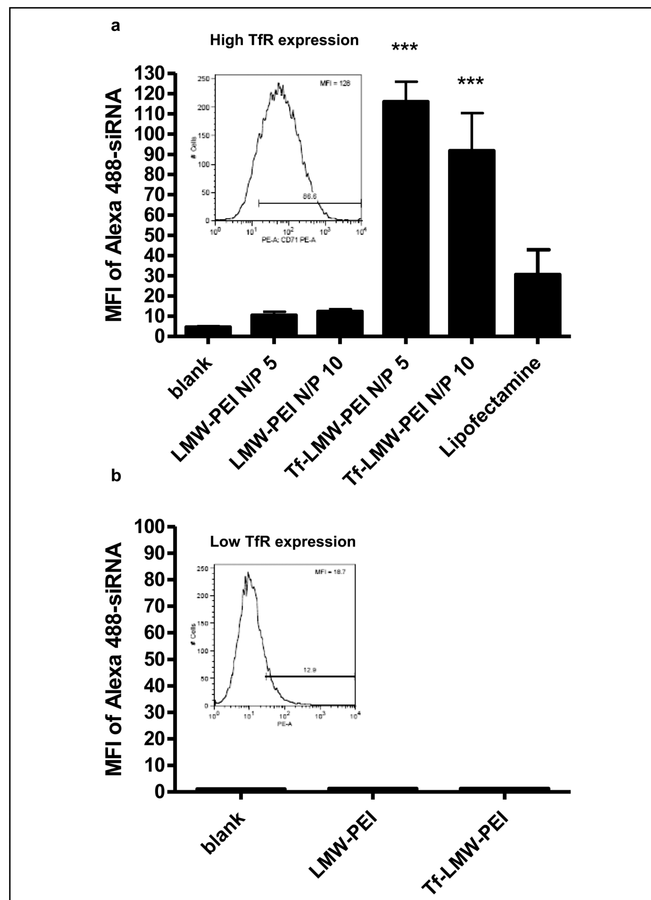


**Figure 2.**

Specific uptake of siRNA in activated T cells with a Tf-PEI conjugate. Uptake of Alexa488-labeled siRNA a) at different N/P ratios into fully activated T cells with high TfR expression (inset in 2a), and b) lack of uptake into T cells with low TfR expression (inset in 2b). The expression of TfR in the T cells was confirmed by anti-CD71 antibody binding assay. The siRNA taken up into the T cells was analyzed by flow cytometry. Lipofectamine was used as a positive control.