

# Reprogramming T cells: the role of extracellular matrix in coordination of T cell activation and migration

Michael L Dustin\* and Antonin R de Fougères†

The stable immunological synapse between a T cell and antigen-presenting cell coordinates migration and activation. Three-dimensional collagen gels transform this interaction into a series of transient hit-and-run encounters. Here we integrate these alternative modes of interaction in a model for primary T cell activation and effector function *in vivo*.

## Addresses

\*The Molecular Pathogenesis Program, Skirball Institute of Molecular Medicine and the Department of Pathology, New York University School of Medicine, 540 First Avenue, New York, NY 10016, USA; e-mail: dustin@saturn.med.nyu.edu

†Biogen Incorporated, 12 Cambridge Center, Cambridge, MA 02142, USA; e-mail: tony\_de\_fougères@biogen.com

Correspondence: Michael L Dustin

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## Abbreviations

APC antigen-presenting cell  
ECM extracellular matrix

## Introduction

T cell activation is a complicated process that in most cases leads to successful host defense but also can lead to disease through pathogen escape or autoimmunity. In order to study this process, it is reasonable to start with a simple system. One of the simplest stimuli for T cell activation is the combination of a single type of adhesion molecule and a single type of MHC–peptide complex freely moving on a two-dimensional supported planar phospholipid bilayer. Under these conditions — but also confirmed when using B cells as antigen-presenting cells (APCs) — the T cell engages in a natural cellular response that is revealed through the supramolecular organization of the interface: the formation of an immunological synapse [1–3,4\*\*]. A ring of engaged MHC–peptide complexes surrounds an initial adhesive plaque, connecting the T cell and APC [4\*\*]. This pattern soon inverts to form a stable ‘bull’s eye’, with the MHC–peptide complexes in the central supramolecular activation cluster surrounded by a ring of adhesion in the peripheral supramolecular activation cluster [2,4\*\*]. Therefore, it is in the T cell’s nature — that is, in its basic programming — to form an organized immunological synapse through a process of receptor engagement, segregation and signaling [5–7].

Recent studies have raised important questions about how the environment of the T-cell–APC interaction impacts on this program of immunological synapse formation [8,9\*\*]. An emerging key to this concept of environmental control is the extracellular matrix (ECM), which is the structural

underpinning of tissues and the primordial environment for evolution of our immune system [10]. Some *in vivo* environments appear to nurture prolonged immunological synapse formation [11] whereas others may re-program the T cell to favor a brief interaction with the APC that can be repeated with many partners in a short span of time [9\*\*]. In this review, we discuss the impact of the ECM on *in vitro* and *in vivo* T cell responses. We also propose a model that integrates immunological synapse formation with what we know about ECM distribution in body tissues, to coordinate antigen recognition and T cell migration in a tissue-specific manner.

## T cells encounter different extracellular-matrix components in peripheral and lymphoid tissues

T lymphocytes are faced with two dramatically different types of environments *in vivo* with respect to the organization of the ECM: peripheral tissues and lymphoid tissues. The peripheral tissues are bounded by epithelium with basement membranes rich in collagen and laminin; the intercellular spaces are largely filled with abundant collagen fibrils (which, in the dermis for example, make up 75% of the dry weight). During inflammation, mediators such as IL-1 trigger increased collagen production by stromal cells [12]. Therefore, T cells that enter the parenchyma of the skin or solid organs are in continuous contact with collagen fibers and other diverse matrix proteins. This is in stark contrast to the organization of secondary lymphoid tissues, where collagen fibrils are sheathed in the fibroblastic reticular cells of the reticular fiber network [13]. Because of this, T cells in the parenchyma of a lymph node or T cell area of the spleen are in a densely packed cellular environment with little ECM.

The major function attributed to the reticular fiber networks of the lymph nodes and spleen has been the transport of solutes from the afferent lymph to the high endothelial venules [14\*]. However, it is notable that the effective masking of collagen fibers from T cells in the parenchyma of lymph nodes makes the secondary lymphoid tissue environment essentially unique. The potential significance of this unique organization is emphasized by a recent *in vitro* study on the effect of collagen fibers on T-cell–APC interactions [9\*\*].

## Interactions of lymphocytes within collagen gels *in vitro*

Wilkinson and colleagues [15] were the first to note that collagen gels stimulate migration of lymphocytes. Recently, Friedl and colleagues [9\*\*] have examined antigen-presentation in collagen gels, with a striking finding that naïve T cell interactions with dendritic cells were dynamic and short-lived (median = 6–12 minutes). Importantly, the serial encounters of naïve T cells and APCs were integrated over

time so that T cells were fully activated without forming a stable immunological synapse. A naïve T cell that encounters antigen in a collagen-rich tissue will have to interact with many antigen-positive APCs sequentially before exiting the tissue in order to become fully activated. We feel that it is most likely that a naïve T cell faced with this situation will exit the tissue prior to reaching sufficient cumulative levels of interaction in order to ensure proliferation.

How can this finding be integrated with immunological synapse formation? We disagree with the conclusion of Friedl and colleagues — that immunological synapses do not form *in vivo* because of the ubiquitous nature of collagen fibers. In contrast, we interpret these findings as supporting a model in which immunological synapses may form readily in T cell areas of secondary lymphoid tissues, where collagen is sequestered in reticular fibers. This stable mode of interaction would ensure that naïve T cells would have sufficient contact time with an antigen-positive APC to provide for a sensitive response in this specialized environment and a much less sensitive response in the periphery. Effector cells that enter the collagenous tissues following primary stimulation in lymph nodes can then engage in serial interactions in the tissues. These serial interactions may be ideal for efficient effector function. Therefore, in our view the environment is likely to have a profound influence on the association of antigen recognition and T cell migration, with different outcomes for naïve and effector T cells. An important point is that naïve T cells express the homing molecules L-selectin, LFA-1 and CCR7 such that they extravasate almost exclusively in secondary lymphoid tissues, where collagen is sequestered from interactions with APCs. In contrast, effector T cells lose L-selectin and CCR7 and increase expression of many adhesion molecules and chemokine receptors (e.g. CCR5) such that they extravasate in inflamed tissues, where collagen is not sequestered from interactions with APCs. Therefore, the effect of collagen may be similar on naïve and effector T cell interactions but the *in vivo* environments of these two cell types are very different.

The mechanism by which collagen fibers alter the naïve T-cell–APC interaction is not clear but there are significant data on the interaction of T cells with ECM proteins — both physically and functionally. T cell interaction with ECM proteins such as collagen, laminin and fibronectin has been shown to be important in T cell adhesion, migration and activation [16]. Although attachment of T cells to immobilized ECM molecules alone does not lead to T cell activation, co-ligation of the TCR with ECM receptors (using anti-CD3 monoclonal antibodies and fibronectin) enhances T cell proliferation. Interactions of T lymphocytes with immobilized ECM proteins occurs largely through a subfamily of receptors — the  $\beta$ 1 integrins [17].

### The role of integrins in T cell migration and activation

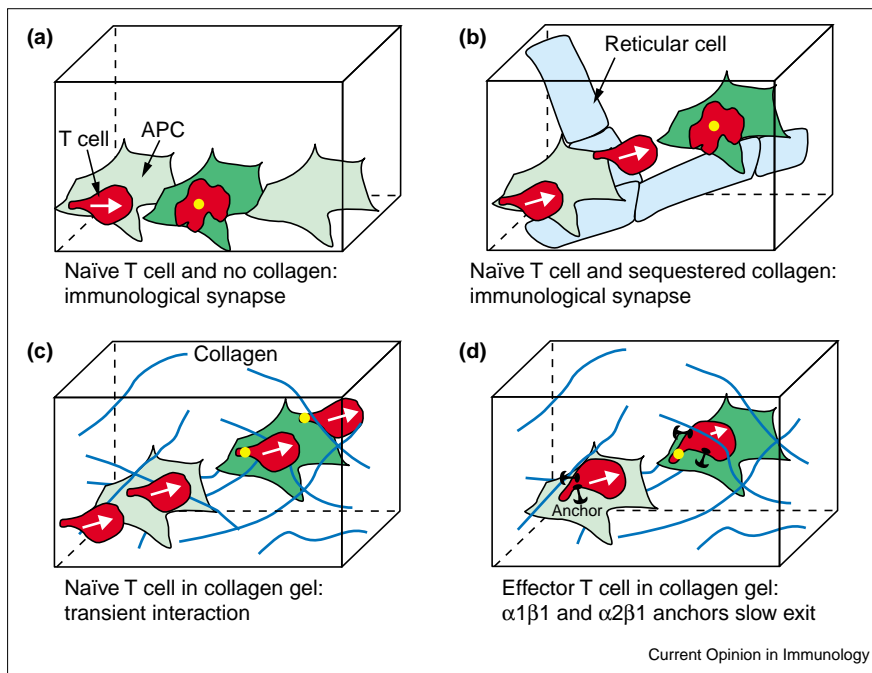
Integrins are noncovalent heterodimers that are expressed on the surface of most nucleated cells, where they form a

transmembrane linkage between specific ECM or cell surface ligands and the cytoskeleton [18]. The  $\beta$ 1 integrins expressed on naïve lymphocytes interact with fibronectin ( $\alpha$ 4 $\beta$ 1 and  $\alpha$ 5 $\beta$ 1 integrins) and laminin ( $\alpha$ 6 $\beta$ 1); all three of these integrins continue to be expressed, at higher levels, on activated T cells. The major cell surface receptors for collagens are the  $\alpha$ 1 $\beta$ 1 and  $\alpha$ 2 $\beta$ 1 integrins [17]. Consistent with the initial description of  $\alpha$ 1 $\beta$ 1 and  $\alpha$ 2 $\beta$ 1 as ‘very late antigens’, activated T cells can express these receptors; such cells include infiltrating T cells in a variety of chronic inflammatory settings but not naïve T cells [17,19–21]. *In vitro*, T cells only express significant amounts of  $\alpha$ 1 $\beta$ 1 and  $\alpha$ 2 $\beta$ 1 after a couple of days stimulation, when the cells take on effector functions. As predicted by the restricted expression of  $\alpha$ 1 $\beta$ 1 and  $\alpha$ 2 $\beta$ 1, Friedl *et al.* [22] have found that migration of naïve T cells through three-dimensional collagen matrices is largely integrin-independent, involving highly transient interactions with collagen that lack the typical focal adhesions and matrix re-organization seen in cell types that express  $\alpha$ 1 $\beta$ 1 and  $\alpha$ 2 $\beta$ 1. Recently, other cellular receptors for collagen have been described, including two novel integrin molecules,  $\alpha$ 10 $\beta$ 1 [23] and  $\alpha$ 11 $\beta$ 1 [24], and two nonintegrin discoidin-like receptors, DDR1 and DDR2 [25,26]. At present, none of these new collagen receptors has been examined for expression on immune cells; thus, although there are certainly candidates for collagen receptors on naïve T cells, none is definitively identified.

Strong evidence for a role of  $\alpha$ 1 $\beta$ 1 and  $\alpha$ 2 $\beta$ 1 in T cell costimulation was obtained using effector T cells [27]. Although attachment to collagen alone did not result in T cell proliferation, TCR-mediated proliferation and cytokine secretion were synergistically enhanced by  $\alpha$ 1 $\beta$ 1- and  $\alpha$ 2 $\beta$ 1-dependent attachment to collagen. A recent report has extended these results to human effector T cells, showing that co-immobilization of TCR with type I collagen results in synergistic T cell activation and proliferation that is dependent on  $\alpha$ 1 $\beta$ 1 and  $\alpha$ 2 $\beta$ 1 integrins [28]. Besides a proliferative role, engagement of the  $\alpha$ 2 $\beta$ 1 integrin, but not  $\alpha$ 1 $\beta$ 1, has also been found to reduce activation-induced cell death in T cells by inhibiting Fas ligand expression [29]. In none of these cases has the effect of collagen on T cell migration been examined. Thus, it is not clear if  $\alpha$ 1 $\beta$ 1 or  $\alpha$ 2 $\beta$ 1 stimulate or inhibit migration or, conversely, immunological synapse formation. In other cell types,  $\alpha$ 1 $\beta$ 1 and  $\alpha$ 2 $\beta$ 1 are used for contracting collagen gels and other functions that require a highly avid interaction that actually slows or stops migration [30]. Thus, these collagen-binding integrins, in contrast to putative collagen receptor(s) on naïve T cells, may actually slow the progress of effector T cells through tissues, to provide the appropriate combination of serial encounters and antigen-specific retention of T cells in the tissues. The  $\alpha$ 1 $\beta$ 1 and  $\alpha$ 2 $\beta$ 1 integrins, by promoting attachment to collagen, may allow T cells to be retained within tissues and be activated in a permissive environment.

The importance of collagen–integrin interactions in modulating *in vivo* inflammatory processes has been described recently [31]. Treatment of wild-type mice with

Figure 1



Model for the role of collagen in coordination of migration and antigen recognition. Antigen is shown in yellow, APCs are shown in light green (i.e. without appropriate antigen) or dark green (i.e. with appropriate antigen) and T cells are shown in red. White arrows within T cells indicate movement of cells whereas T cells with 'bull's eye' patterns have stopped and are forming immunological synapses.

(a) If no collagen is present or (b) collagen is sequestered in reticular cells (light blue), naïve T cells can form a synapse with APCs bearing the appropriate antigen. (c) In the presence of a collagen gel (dark blue) *in vitro*, interaction between naïve T cells and APCs is transient and incomplete whereas (d) the expression of  $\alpha 1\beta 1$  and  $\alpha 2\beta 1$  on activated T cells means that the T cells are anchored to the collagen; this may enable prolonged interactions with APCs.

monoclonal antibodies (mAbs) to either  $\alpha 1\beta 1$  or  $\alpha 2\beta 1$  was found to inhibit significantly effector-phase inflammatory responses in models of both contact and delayed-type hypersensitivity; anti- $\alpha 1$  mAb treatment also suppressed development of arthritis. Likewise,  $\alpha 1$ -deficient mice also show decreased inflammatory responses in models of contact hypersensitivity and arthritis. As expected by the restricted expression of  $\alpha 1\beta 1$ —on activated immune cells— $\alpha 1$ -deficient mice show few immune defects in the unchallenged state [32]. Thus, whereas putative collagen-binding receptors on naïve T cells may promote migration and thereby restrict immunological synapse formation to collagen-free environments (i.e. secondary lymphoid tissues), the collagen-binding receptors expressed on activated T cells— $\alpha 1\beta 1$  and  $\alpha 2\beta 1$ —may help to retain effector T cells in antigen-positive collagenous tissues, allowing T cell activation (Figure 1).

The exact mechanisms by which integrins and other collagen-receptors are linked to specific intracellular signaling pathways that result in appropriate cellular responses are not well studied in leukocytes although in other cell types distinct differences have been found among different integrins, including  $\alpha 1\beta 1$  and  $\alpha 2\beta 1$  (reviewed in [33]). In addition to differences in downstream signaling pathways, the two main collagen-binding integrins differ in the structural recognition of their ligands; although  $\alpha 1\beta 1$  and  $\alpha 2\beta 1$  have relatively small differences in their structure, these two integrins differ in the binding specificity for collagen subtypes, with  $\alpha 1\beta 1$  showing a preference for type IV collagen and  $\alpha 2\beta 1$  showing a preference for type I collagen (reviewed in [34]).

Identification of the  $\alpha 1\beta 1$ - and  $\alpha 2\beta 1$ -binding sites in collagen revealed a GFOGER sequence (single-letter code is used for amino acids) in native triple-helical collagen that represents the high-affinity binding site in type I collagen for both  $\alpha 1\beta 1$  and  $\alpha 2\beta 1$ , and in type IV collagen for  $\alpha 2\beta 1$  but not  $\alpha 1\beta 1$  [35\*,36]. Currently, 19 genetically distinct collagen types exist that can form different higher structures (i.e. fibrils, networks and beaded filaments) and can be expressed in different anatomical locations (i.e. type I is extravascular, type IV is basement-membrane-associated). The structure and type of collagen expression in tissues is also known to change in pathological conditions, especially in fibrotic diseases such as scleroderma [12,37]. The collagen gels employed by Friedl and colleagues [9\*\*] were of type I collagen, raising the question of how different collagen types will affect naïve T cell responses. Thus specificity of collagen interaction with cellular receptors can take place both at the level of integrin expression and the collagen type. How these different combinations of ECM–integrin interactions influence TCR synapse formation and cell activation remains to be determined.

## Conclusions

We conclude that T cells have a basic program to form an immunological synapse when a threshold level of TCR signaling is achieved. The stability of these structures may be impacted by factors such as co-stimulation, chemokine gradients and, as we have discussed here, collagen ECM. This environmental control of immunological synapse formation and T cell activation by collagen may have a profound influence on peripheral tolerance and T cell effector functions that are relevant to tolerance, autoimmune disease and tumor immunity.

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