

Dancing with Integrins

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The 2019 Canada Gairdner International Award recognizes Timothy Springer's discovery of the first immune system adhesion molecules involved in lymphocyte homing and the translation of those discoveries into therapeutics for autoimmune disease and cancer. It also celebrates a master communicator who used choreography to explain integrin activation to new audiences.

The cells of the immune system are remarkable in their ability to gracefully travel throughout the body and cross boundaries to maintain healthy tissues and protect the host from pathogens. Immune cells can also under-react to pathogens and tumours and overreact to otherwise healthy tissues leading to urgency to develop methods to boost or suppress immune cell function. One way to do this is by controlling their access to tissues. The study of cell adhesion in the immune system has paralleled work in other tissues and organs, and has often taken the lead in establishing important concepts in addition to providing new therapeutic targets. Over the past 40 years, Timothy Springer's contributions to this effort include identification and functional characterization of adhesion receptors (Springer, 1990), elucidating the multistep model for leukocyte extravasation (Springer, 1994), and the shape shifting mechanisms that regulate the function of a major group of adhesion receptors, the integrins (Luo et al., 2007). Springer's 1990 review (Springer, 1990) was photocopied so many times that the pages in the hardbound copy at the Countway Library in Boston were completely worn away. The key discoveries recognized by the Canada Gairdner Prize focus on how immune cells travel through the blood stream and can enter different tissue sites using molecular area codes. Limiting cell use of specific area codes was seen as a pathway to treatment for a wide range of human ailments. This benchmark discusses the scientific context of Dr. Springer's fundamental scientific exploration and efforts to effect a reduction of human suffering.

There are two contexts in which to view Dr. Springer's Benchmark discovery celebrated by this Prize: the broad effort to understand cell adhesion across all of biology and the more specific field related to how immune cells navigate the body at a level of detail needed to design a drug. To create an outline for this Benchmark essay, it's helpful to review what a leukocyte needs to do to leave the blood at a site of inflammation (Figure). Vascular hydrodynamics dictates that leukocytes attach to blood vessels in post-capillary venules, but even in these sites the flow rate is measures in many cell diameters per second, requiring mechanism that can "hook" a cell quickly when-ever a leukocyte is in contact with the vessel wall. These initial interactions lead to "rolling adhesion" that involved rapid formation and breaking of these molecular hooks. Then the leukocyte can either release from the surface and go around the circulatory loop again or arrest the rolling, firmly attach to the vessel wall and crawl out through or between the endothelial cells into the tissue. This process was well described over many years but the molecular elements that were needed to understand how leukocytes enter tissues from the blood were identified by Dr. Springer and colleagues during the 1980's and Dr. Springer was able to put these together into a coherent model in 1991.

It could be argued that Dr. Springer came up with the first definitive data in support of adhesion receptors in any system- based on purified cell surface molecules with clearly demonstrated ability to mediate cell adhesion under physiological conditions. Other systems that were being described in the same time frame suffered from complications with purification and measurement of activity. Neural cell adhesion

molecule (NCAM) (Cunningham et al., 1987) was one of the better characterized adhesion systems in this period, but even this exemplar faced many complexities establishing how the low affinity interactions work to mediate functional adhesion in the nervous system. There were often ambiguities between what was a direct interaction and what was regulation of other interactions. There were some advantages of studying cells of the immune system- which were “non-adherent” cells that interacted with other cells only in certain conditions and then only transiently. It was also an advantage that most of these adhesion systems were heterophilic- meaning that distinct molecules on the different cells mediated the cell-cell interaction.

Dr. Springer had a head start on monoclonal antibody (mAb) technology and was among the first to recognize the power of unbiased functional screening of monoclonal antibodies libraries elicited by immunization with complex immunogens. This approach presented the first access to the wider “proteome” of the cell surface. The putative receptors identified could be purified by immunoaffinity chromatography with the ability to carry out >100,000-fold purifications in one step. While many investigators were focused on T cell receptors (TCRs), Dr. Springer made the interesting choice of using polyclonal cytotoxic T cell lines for a functional screen for antigen specific killing, with many different TCR and thus stayed away from the pack that was battling over the TCR (McIntyre and Allison, 1983) and discovered lymphocyte function associated antigens (LFA)-1, -2 and -3 (Sanchez-Madrid et al., 1982), which defined the two major adhesion mechanisms important for T cell cytotoxicity, one of which was also critical for leukocyte trafficking.

LFA-2 and 3 are important adhesion molecules, but LFA-1 turned out to be a keystone in building an understanding of regulated lymphocyte adhesion and trafficking. The LFA-1 adhesion receptor is a heterodimer and one set of mAb recognized only LFA-1 and the other recognized three heterodimers that included Mac-1, and another receptor called p150,95. Dr. Springer was the first to determine that there was a family of three (now known to be four) leukocyte specific heterodimers that had distinct large (α) subunits that shared a common smaller (β) subunit and that mutations in the common β subunit resulted in leukocyte adhesion deficiency-1 (LAD1) (Kishimoto et al., 1987a). The leukocytes from these patients had profound defects in interaction with the vessel wall at sites of inflammation and the patients died of bacterial infections during childhood unless treated by allogeneic bone marrow transplantation.

Cells from LAD1 patients were instrumental in identifying the receptors that act as binding partners for LFA-1. While LFA-1 deficient leukocytes cannot aggregate with themselves, they could co-aggregate with LFA-1 expressing cells from healthy individuals. Immunization of mice with cells from LAD1 patients generated a library from which one new adhesion blocking mAb was selected-intercellular adhesion molecule-1 (ICAM-1). The partnership of LFA-1 with ICAM-1, and LFA-2 and LFA-3, were among the first and best defined adhesion systems and led to a new field of studying lymphocyte adhesion (Springer, 1990), but there were still many gaps.

Quantitative adhesion assays performed with the cultured endothelial cells suggested that there was at least one other LFA-1 partner and there was also at least one LFA-1 and ICAM-1 independent adhesion mechanism (Dustin and Springer, 1988). Dr. Springer cloned the second LFA-1 binding partner, which was highly expressed on endothelial cells in the steady state called ICAM-2. Dr. Springer had actually already made antibodies to the second LFA-1 independent adhesion system, in the same mAb screens that identified LFA-1, -2 and -3, but the function connect had not yet been made.

Lymphocytes expressed another set of large heterodimers referred to as “very late activation” (VLA) antigens as several of them were expressed only on highly differentiated effector and memory T lymphocytes, but the connection to leukocyte trafficking was not yet clear. Springer published the sequence of the β subunit of LFA-1, Mac-1 and p150,95 in 1987 (Kishimoto et al., 1987b). This was a rare situation where being second made the result even more important. A year earlier, Richard Hynes had published the sequence of one of the subunits of the fibronectin receptor, referred to as integrin (Tamkun et al., 1986). Integrin had a high level of similarity to the LFA-1 β subunit leading to the definition of the integrin family: the fibronectin receptor β subunit becoming $\beta 1$, the LFA-1, Mac-1, p150,95 β subunit becoming $\beta 2$, and the platelet glycoprotein IIb/IIIa β subunit becoming $\beta 3$. We are now up to $\beta 8$ and there are 18 α subunits in the integrin family. The VLA antigens identified by Dr. Springer in the original screen turned out to be other members of the $\beta 1$ integrins! Biogen identified VLA-4 as a receptor for the vascular cell adhesion molecule (VCAM) and this was the LFA-1 independent adhesion pathway mentioned above (Ellices et al., 1990). This turned out to be an important pathway for entry of lymphocytes into the central nervous system and the anti-VLA-4 antibody natalizumab is still an important drug for treatment of rapidly-evolving severe relapsing-remitting multiple sclerosis. However, it was still not clear how leukocyte entry into tissues worked.

Understanding the steps through which leukocytes attach to vessel walls required another type of adhesion molecule. Mel-14 on leukocytes, PADGEM on endothelial cells and platelets and ELAM on endothelial cells are members of the selectin family (also called CD62L, P and E). These molecules were candidates for partnering with integrins like LFA-1 and Mac-1 in supporting leukocyte adhesion to the vessel wall from flow. In vivo, antibodies to L-selectin (also called LECAM) blocked the rolling component of adhesion, whereas antibodies to integrin $\beta 2$ blocked the firm adhesion (von Andrian et al., 1991). Dr. Springer reconstituted the entire process in vivo using purified molecules (Lawrence and Springer, 1991). He was able to demonstrate that purified P-selectin mediated rolling adhesion, but no firm adhesion was established even when ICAM-1 was included under flow conditions. There was still something missing from this two-step model. Only when the leukocytes were exposed to a chemotactic peptide emulating the N-terminus of a bacterial protein binding to a GPCR, did the rolling adhesion on P-selectin transition to firm adhesion due to the concurrent activation of LFA-1 and Mac-1 to bind ICAM-1. These three steps—selectin mediated rolling, GPCR mediated activation and integrin mediated firm adhesion—make up the three distinct digits of the leukocyte adhesion code.

In the mid 1990's Dr. Springer learned structural biology— a remarkable mid-career retraining. In his inaugural paper after induction into the US National Academy of Science, Dr. Springer published a structural prediction that 7 repeats in the integrin α subunits would fold into a 7 bladed β propeller domain (Springer, 1997), which was confirmed by the first complete crystal structure of an integrin. However, an equally important in some respects more unique dataset was generated by Dr. Springer and colleagues using electron microscopy, in which entire purified integrins were visualized through class averages of thousands of examples with ligand or various antibodies bound (Takagi et al., 2002). The crystallographic information could then be laid out on the native skeletal structures arising from the electron micrographs to develop better models for the global conformational changes leading to atomic changes that controlled ligand affinity and accounted for their sensitivity to signaling from GPCRs. Dr. Springer described the structural elements and conformational changes using lively metaphors that invoked action and dynamics.

Springer was awarded the Crafoord Prize by the Royal Swedish Academy of Sciences in September of 2004 and for this occasion commissioned a modern dance interpretation of integrin activation called "Turning on Integrins" (<https://vimeo.com/143275802>). Springer elevated science communication to art. Thus, the title of this Benchmark!

To further translate these findings to improve human health, Dr. Springer started the company Leukocyte. Leukocyte developed an antibody to a novel integrin with the α subunit of VLA-4 paired with $\beta 7$, an integrin heterodimer which binds to mucosal addressin cell adhesion molecule (MAdCAM), a surface molecule expressed in the mucosal vasculature. This antibody, vedolizumab blocks entry of T cells into mucosal sites and is approved for treatment of ulcerative colitis and Crohn's disease. Leukocyte was purchased by Millenium, and then Millenium by Takeda before approval of the drug by the FDA in 2014. Thus, Springer's research and entrepreneurial activities set in motion new therapies for autoimmune disease, reducing human suffering and realizing the promise of the multistep paradigm.

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