Interview

Immunotherapeutic manipulation of the tumor microenvironment

Dario AA Vignali speaks to Katie Lockwood, Assistant Commissioning Editor

Dario Vignali received his PhD in 1988 from the London School of Hygiene and Tropical Medicine, University of London, where he studied immunity to *Schistosoma mansoni*. He then held two postdoctoral positions from 1988 to 1993; first at the Institute for Immunology and Genetics, German Cancer Research Center (Heidelberg, Germany), with Gunter Hammerling, and then at the Department of Biochemistry and Molecular Biology, Harvard University (MA, USA), with Jack Strominger. He is currently Vice Chair and Member (Full Professor equivalent) of the Department of Immunology, St Jude Children's Research Hospital (TN, USA), where he has been for 20 years. Dr Vignali's research focuses on molecular and cellular aspects of Treg function, immune regulation by inhibitory receptors and inhibitory cytokines (IL-35) in tumor immunity, mucosal immunity and Type 1 diabetes. He also studies proximal events in T-cell receptor-CD3 signaling.

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■ **When did you first become interested in immune regulation?**

The reality is that my interest probably started very early on in my career, although that obviously changed with time. I became interested in immunology during my degree, but I became particularly interested in immune regulation during my PhD because I was working on immunity to tropical parasites. In that situation, immune regulation is critical to maintain the balance between the pathological consequences of the pathogen, but also the excessive consequences of immune pathology that often occur in chronic infections. That dynamic has always been of interest to me and after starting my laboratory here at St Jude Children's Research Hospital, we became interested in the opposing regulatory environment that occurs in autoimmunity versus the tumor microenvironment. In autoimmunity you have regulatory insufficiency as a consequence of either defective Treg and inhibitory receptor control or resistance to immune regulation by the effector T cells. In cancer it's the opposite; you have excessive immune inhibition, either through upregulation of inhibitory receptor pathways or through Treg-mediated suppression. We are entering an exciting period as these pathways are being successfully targeted therapeutically, with quite remarkable observations made with ipilimumab (anti-CTLA4) and nivolumab (anti-PD1).

Therefore, this area has become interesting not only pharmacologically, but immunotherapeutically.

■ **With regard to your recent work, what prompted you to investigate the Sema4a & Nrp1 pathway in Tregs?**

We have a long-standing interest in understanding Treg control, as have many other groups. However, we've always been particularly interested in their activity in highly inflammatory sites or their dysfunction in autoimmunity. Consequently, we were interested in trying to identify pathways that are defective in autoimmunity and exacerbated in the tumor microenvironment. However, in contrast to many people who have been trying to identify molecules that mediate Treg development and general immune homeostasis (i.e., general proteins and pathways required for their immune maintenance), we have been more interested in those molecules and pathways that are selectively used in inflammatory sites, such as the tumor microenvironment. This is because Tregs are very potent immune regulators and we know that their complete elimination can have devastating consequences, as exemplified in immunodysregulation polyendocrinopathy enteropathy X-linked syndrome patients, who possess inactivating point mutations in Foxp3, a transcription factor required for

Treg development. This gives rise to a lack of Tregs and these patients will die fairly early on in life unless they receive a bone marrow transplant. This illustrates the dangers of completely eliminating Tregs, while at the same time illustrates their therapeutic promise when their activity is transferred or enhanced. We obviously cannot target pathways that are required for their general immune function because of the potential adverse consequences of autoimmunity. Therefore, we wanted to determine whether there were pathways that were selectively used in tumors, that wouldn't be required for their general immune maintenance. That was the starting point, and whenever you have a discovery-initiated program the first question is 'can we develop a simple *in vitro*-based screening assay to identify those pathways that might be most active in that kind of situation?' So that question was what prompted this study.

■ **As this pathway is not involved in general immune maintenance & there are no unwanted autoimmune reactions, is it dispensable?**

The pathway seems to be entirely dispensable as mutant mice do not develop any autoimmune or inflammatory reactions.

■ **Did you see any interference in the stability of the Treg population?**

Well we did, but only in the tumor micro-environment, not in the periphery. The caveat here, and with any first study, is that we obviously didn't look at every disease model or scenario and it remains to be determined whether or not this pathway may contribute to exacerbating a pre-existing autoimmune condition. We didn't look at that in this study. All that we can say is that without receiving any external or genetically induced insults that might enhance inflammation or autoimmunity, the mice don't suffer any consequences. However, their capacity to clear tumors is substantially enhanced. Although it's evident from the data that the extent to which this pathway is used may not necessarily be the same in every tumor model. Indeed, in the three tumor models we used, there was clearly an effect in all three, but it was variable, with the

Nrp1-mutant mice demonstrating complete resistance to a melanoma model, while only showing delayed tumor growth with the adenocarcinoma model.

■ **Do you think there might be other pathways, similar to this one, which could also be selectively targeted in tumors without having any unwanted side reactions?**

Yes, and indeed we are interested in this question. This study was, at one level, a proof-of-principle, so this is the first example that I'm aware of, of a pathway that functions in this way. However, that does not preclude the possibility that there could be other pathways that may coexist, although, we speculate that may not be likely, simply because of the Treg fragility observed when this pathway was removed in the melanoma model. However, maybe the reason we didn't see a big effect in the MC38 model was because there really are other pathways that are more important in those tumors. Different tissue sites may also dictate differential utilization of different pathways, and of course we still need to determine whether this is a dominant pathway within human Tregs.

■ **Do you have any plans for further studies using human tissue?**

Yes. We're interested in determining how important this pathway is in human Tregs and whether there are other pathways that may be more important in human Tregs.

■ **You mentioned that the effect of targeting the pathway was greatest in a melanoma model, is that going to be a focus for the future or are you going to continue to investigate a range of cancer models?**

The answer to that is actually a bit of both. We, like most tumor immunologists, are particularly interested in melanoma because it's one of the most immunologically impacted tumors. There's a long history of substantial immunological intervention in melanoma, but with very potent immune inhibition that prevents tumor clearance. Thus it's not coincidental that many, if not most, of the new immunotherapies targeting inhibitory pathways in Phase I/II trials include melanoma. Therefore, we are

particularly interested in melanoma for that reason, not only on the human side, but also in mouse models of melanoma. However, we are also very interested in investigating the potential impact of the Nrp1/Sema4a pathway in other tumor types, particularly lung and gastrointestinal tumors.

■ **You also have research interests in Type 1 diabetes, is there anything you've learnt from Tregs in cancer that can be translated to Type 1 diabetes?**

That's something we're actually focusing on the moment. One hypothesis is that a pathway that is particularly important in maintaining optimal Treg function in a tumor may be a pathway that is defective, or not utilized, in an autoimmune setting. The first question for us, is 'whether this pathway is used by Tregs in autoimmune mouse models of Type 1 diabetes and multiple sclerosis.' We, like many in the Type 1 diabetes field, use the NOD mouse model, as it spontaneously develops autoimmunity and shares some of the hallmarks of the human condition. We generated NOD mice with a selective Treg defect in Nrp1 and are currently asking whether or not this pathway is utilized. We're at a very early stage, so we don't have any information yet, but the questions are obvious; is the pathway used, and if it isn't, what are the mechanisms that are preventing it from being used? Of course, all of this raises the possibility that we may be able to therapeutically enforce the utilization of the Nrp1/Sema4a pathway and perhaps make Tregs more stable, which, in turn, may potentially limit autoimmunity. That's all ongoing and to be determined.

■ **Do you have any words of inspiration to anyone going into a career in this area, or in immunology in general?**

From my perspective, immunology is one of the most fascinating subjects because it has the power to both create and mediate diseases that are devastating (e.g., if somebody with a peanut allergy ingests even the

slightest amount of peanut they could die very rapidly). However, at the same time, the immune system has the amazing power to cure. For instance, we can vaccinate people against something that would otherwise be lethal, such as yellow fever, and induce complete immunity. That shows that the immune system has great power to do good or to do harm. Therefore, it presents both a biological and a potentially therapeutic interest, in terms of trying to understand how things work at the basic mechanistic level and then determine how that could be utilized to create effective therapeutics. While not a new area, I think this is a particularly exciting time because we are now developing tools that are more powerful and generating more information than we've ever had before. The long-held promise of targeting the immune system to generate new therapeutic approaches in both inflammatory and autoimmune disease, and also in cancer are now starting to bear fruit. Therefore, for anyone interested in biomedicine, this is the perfect time to enter immunology and immunotherapeutics. Whether you are interested in pursuing laboratory-based science, clinical-based science or even becoming a physician, there are numerous opportunities and questions. There's room for everybody.

Disclaimer

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