Regeneration Biology
Rajesh Ramachandran
Department of Biological Sciences
IISER Mohali
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W8L40_Dynamics of immune system in organ regeneration

Hello everyone, welcome back to another class on regenerative biology, and in today's class, we will learn about the dynamics of the immune system in organ regeneration. Like in the previous class, we learned about the involvement of Tregs, and in today's class, we will learn a lot more about the Tregs from a regeneration point of view. And it should be clearly understood now that regeneration is not a simple cellular or tissue-level event; it also depends on the physiology of the animal, and it also depends on how efficiently the animal or that given tissue of that animal is able to deal with immune cells. So this is very important to follow up. Let us see some examples. Examples can be from different species and different animals.

Zebrafish can efficiently regenerate complex tissue structures with a highly developed innate and adaptive immune system. Zebrafish have a perfect immune system. immune system to deal with all these scenarios, which provide a model to investigate the roles of the immune system or immune cells during tissue repair and regeneration. So zebrafish not only have that regenerative capacity, but it has got a well-developed immune system.

In other words, if you find some uniqueness in mammals' immune systems, you will automatically find a homolog. Say you are talking about Treg cells. You are talking about granulocytes, or you are talking about monocytes. You have similar cells in zebrafish. So that makes it an attractive organism to explore from the immune angle as well.

Two groups of researchers have recently reported zebrafish mutants that are deficient in the forkhead box P3 or foxp3 ortholog, which helped to reveal the conserved immunosuppressive function of zebrafish foxp3 in vivo. So FOXP3 is a functional marker of Tregs. Or in other words, if FOXP3 is missing, regulatory T cells are useless or they are not functional. It's almost like if your hand were missing, you would be there as normal. Your leg is missing, you are there.

What if your head is missing? Your identity is lost; life also is lost. In the same way, FOXP3 is an important gene for regulatory T cells; zebrafish FOXP3 defines the development of a subset of T cell lineage with a conserved gene expression profile of mammalian regulatory T cells. As I told you some time ago, zebrafish have a lot in

common with mammals. In damaged organs, zebrafish T. rex rapidly migrate to the injury site, promoting the proliferation of regenerative precursor cells by producing tissue-specific regenerative factors through a distinct mechanism from the canonical anti-inflammatory pathway.

So Treg cells are essential to be present at the injury site if you want to have a proper regenerative response. So these findings illuminate the potential for using zebrafish as an effective model in Treg research focusing on regulatory T cells and demonstrate the organ-specific roles of Tregs in maintaining pro-regenerative capacity that could potentially be harnessed for use in diverse regenerative scenarios or therapies. So if you look further into the background of the Tregs, it plays essential roles in maintaining tolerance to self-antigens, like how your body does not produce antibodies against your own tissues or proteins. If it does, then that is called an autoimmune disease. If any of your organs become the target of your own immune system, that organ will be damaged, and this Tregs plays a major role; it helps to prevent autoimmune disease and restrain aberrant or excessive immune responses.

to non-self antigens. Self-antigens mean your own proteins, while non-self antigens are at the inflammation site. So an excessive immune response is always problematic. Probably during the COVID season, you may have heard that many of the deceased patients' bloodstreams had high levels of IL-6, interleukin-6. which can trigger a very pro-inflammatory environment and can also cause pneumonia, etc.

So it is important to note that the Tregs allows an adequate inflammatory response even if there is a non-self antigen, such as a bacterium or a virus, etc. So Tregs development and function are controlled by a master regulatory gene that encodes the FOXP3 protein in mammals. Like I told you, Tregs are Tregs because of FOXP3. And the deficiency in FOXP3 function causes a fatal autoimmune syndrome both in mice and humans. If the FOXP3 gene is mutated, both mice and humans will develop autoimmune diseases.

Treg-mediated immunosuppressive mechanisms have been extensively investigated in diverse immunological contexts, but the pleiotropic function of Tregs has increasingly been recognized in recent research studies. In certain mouse tissues, Tregs infiltrate the wound site. Okay, so they are welcomed. They have to come into the wound site, providing canonical immunosuppressive function and paracrine signals that promote the proliferation and differentiation of resident tissue stem cells. It has to; the Tregs has to come to favor this pro-regenerative environment.

And these resident stem cells, along with their proliferation, will facilitate efficient repair or regeneration of damaged tissues because of the paracrine pathways played by the

Tregs. You may have noticed that even if you get a small scratch wound, if your health is not fine, it means you have not been sleeping properly for several days; your immune system gets affected, and that site remains inflamed for a long time. But if you are healthy and perfect, it heals in no time. Simple wound healing is not about regeneration. So the discovery of the regenerative function of Tregs led to an interesting question in regeneration biology: whether Tregs exists in animals with robust regenerative capacity like zebrafish.

Does it have the Tregs? Such as amphibians, urodial amphibians such as axolotls, newts, etc. If so, to what extent does Tregs contribute to organ regeneration? What is their say? Can we address it from that angle? So now people did research, conducted in vivo characterization of the function of zebrafish, FOXP3. There are two FOXP3 orthologues in zebrafish, FOXP3a and FOXP3b. Remember, FOXP3 is essential for Treg formation, function development, and everything. Even the routine function is found to be present in the zebrafish genome 3a and 3b foxp3, and it could play a redundant role; this is the assumption because telios fish have undergone some tetraploidization event around 340 million years ago, which made a diploid organism out of it.

A haploid genome is 23 chromosomes, a diploid is 46, and 46 becomes 92 after division in the S phase of the cell cycle. Now, if 92 became your 2n, then you can tell that you got tetraploidized, so your haploid will be 46 instead of 23; your haploid genome will be 46. Now, if 96 becomes normalized, that is called tetraploidization. Then what will happen is that 46 is a doubling of 23. So, 23 had your entire genome.

Now it has become 46. So you will have a duplication of entire genes. So that has happened for teleost fish. That's why they have duplication of many of these genes. FOXP3 function in the immunosuppressive machinery is evolutionarily conserved in non-mammalian vertebrates, and FOXP3 plays an immunomodulatory role in zebrafish as well.

Mutant zebrafish lines that have a loss of function in foxp3a are available; these mutants have a mutated foxp3 gene and do not have foxp3a alleles, not b alleles. They are used for assessing inflammatory responses in immune cell homeostasis in zebrafish, so homozygous mutant zebrafish mean that foxp3a is missing. 3B is there; 3A is missing. Zebrafish are completely normal during their juvenile stages but exhibit profound infiltration of mononuclear cells in the peripheral tissues with age. Peripheral tissue means outside the boundary of the cell and the boundary of the body.

With age, there is a gradual decrease in growth and survival, including skin, gills, mucosal tissues of the pharynx, and intestine. So we can see this in the picture. So,

analysis of FOXP3A mutants in zebrafish. So this is the wild type and this is the mutant. We know this is 95 days old; that means around three months old.

They are adults, but this is a normal size wild type. This is a little smaller. So we can see FOXP3 wild type has a normal amount in gill skin and pharynx, and there is a normal supply of mononuclear cells; whereas in FOXP3 mutants, there is too much cell infiltration. You can see here that the number of cells is way too high, so the inflammatory cell infiltration is seen in. Treg-depleted or FOXP3-depleted cells.

The mononuclear cells are super abundant without any injury for no reason, and they make it inflamed. The environment is now kept inflammatory, and this is seen with HE staining. And these mutants appear normal, but they are in a highly inflamed scenario. So homozygous zebrafish mutants are entirely normal during their early stages of development. But the infiltration of mononuclear cells is seen across all peripheral tissues.

And with age, as the animal grows older, there is too much infiltration of the mucosal tissues, such as the skin, pharynx, and gills. And there is also this that causes a gradual decrease in the growth of the animal itself. Now, Zebrafish Treg function and its role in organ regeneration are being studied. Zebrafish effectively regenerate their hearts through the proliferation of existing cardiomyocytes. We all know that if you look into heart regeneration.

FOXP3A-positive cells and FOXP3A-expressing cells actively infiltrate the damaged heart from 3 to 7 days after injury, which means Treg cells remain in the damaged myocardium until the regeneration is completed. The peripheral blood, on the other hand, indicates that the heart is injured. Now we are looking into the blood of the zebrafish. FOXP3A-positive cells increase ten-fold after an injury, indicating that the mobilization of FOXP3A cells to the injured side is normal. Why did you find too many cells from three to seven days in the injured heart? Because it is very highly present in the blood due to the injury.

Of course, the injury to the heart has triggered this increase. Otherwise, it will not be present. These are all in normal cells. In the wounded area, FOXP3A positive cells are found within the vicinity of proliferating cardiomyocytes.

Not anywhere else. It is right in the place where the proliferation is occurring. Some of the Treg cells are found to be directly associated with the proliferating cell, which suggests a paracrine route in myocardial regeneration. So these FOXP3 cells migrated into the heart. Anyway, it is present at high levels in the blood and has migrated into the

heart. favors the proliferation of cardiomyocytes.

Sometimes it is in very close proximity, suggesting that the proliferation is influenced by the paracrine function of the Treg cells. After cardiac damage, zebrafish Tregs ingress from the bloodstream. into the regenerating myocardium where the mature Tregs are activated and expand, as opposed to the differentiation of the peripheral Tregs at the injury sites. They migrate from the bloodstream and expand in number.

This is what usually happens. So in this picture, you can see the tissue-specific function of zebrafish Tregs. So sections of injured and uninjured FOXP3A-back-tagged RFP tissue. This is a transgenic line used to detect the Tregs cells physically. You can see no injury, and after that, cardiac injury. And what you are seeing is a gel picture of various genes like NTF, NRG, IGF, and IL-10.

Different genes have been looked at in different tissues. They have looked for kidney cells, spinal cords, hearts, and retinas. They have looked at what happens in normal fish, as well as in Treg-depleted or FOXP3 mutant fish. And here we can see that the control is different; actin is used as a control, and immunofluorescence has been done in the heart for the myosin heavy chain marks, which are seen in the cardiac muscle. Asterisks indicate the center of the injury spot, and the dashed line indicates the outline of the injury in these pictures.

What you are seeing in panel B is the... Picromallory staining of the heart sections from the control left and the T-reg depleted right. That is the FOXP3 mutant that you are seeing here. And see its zebrafish Tregs are purified from the injured and uninjured kidneys, as controlled and analyzed by qPCR, and various genes have been looked for to determine whether the Tregs are migrating to that injured tissue or not. That is why isolated tissue has been used for qPCR. And in panel D, what you see is that zebrafish Tregs promotes organ regeneration by producing tissue-specific regeneration factors.

You can see the injury, spinal cord, heart, and retina. What they see in the spinal cord is NTF3 and IL10, interleukin 10. NTF3 is in the radial glia, IL-10 is for controlling inflammation, whereas in the heart, NRG1 is there for upregulated cardiomyocytes, and it can inhibit the inflammatory response. Also, in the retina, IGF1 is present in the Müller glia and also inhibits inflammation. We can see in the last panel that E is a model for the alteration of Treg function during evolution, so what they are showing here is.

Zebrafish Tregs, which has an extensive regeneration function in evolution, has gone down in the case of mammalian Tregs; when it comes to anti-inflammation, although it is low to start with in zebrafish Tregs, in mammalian Tregsit is quite high, so we can say

mammalian Tregs are less effective. Regenerative in nature, whereas zebrafish T-regs are more regenerative based on their paracrine signaling, and zebrafish T-regs are less anti-inflammatory in function, whereas mammalian T-regs are more anti-inflammatory in function, so we should understand that when it comes to evolution, T-regs have evolved in opposite directions. So if you look further, you will see the Tregs in diverse regeneration contexts. Treg-depleted zebrafish exhibit compromised myocardial regeneration, forming a thinner myocardial wall with striking fibrin deposition. Remember the extracellular matrix? Something similar to that of fibrin; fibrin is a part of the blood clot.

Fibrin deposition and persisting collagenous scar collagen deposition are also present, so when T-regs are depleted, it favors a collagen-depositing environment. Cardiomyocyte proliferation was significantly reduced in the T-reg-depleted heart because we have already seen T-regs are associated with. Proliferating cardiomyocytes. So if FOXP3 is not present, Tregs are not present.

Hence, you don't have too much proliferation. Treg-depleted hearts showed a significant reduction in the expression of specific cardiogenic mitogens such as NRG1, IGF2A, IGF2B, and PDGFB. They are depleted. Cardiogenic mitogens. Zebrafish Tregs express all of these factors in the regenerating hearts, but they express Neuregulin 1 most abundantly. So, Tregs are influential in the proliferation of cardiomyocytes and in other tissues through their paracrine pathway.

Tregs regulate the regeneration of these tissues, which is intriguing because the mechanism by which the spinal cords and retina regenerate is distinct from that of the heart. So we should understand that although Tregs are coming into the vicinity of every damaged tissue, they are not doing the same job in different tissues. So what it tells you, although the paracrine role is there, is that in the target cell it is not causing the same gene expression because we can understand you don't wear the same kind of dress in the office and the same kind of dress on Sunday at home because the ambience is different in the same way. These cells can influence that behavior based on which tissue you are influencing.

Those genes expression will be triggered. Eventually, regeneration happens. In zebrafish, spinal cord and retina regeneration is mediated through the de novo neurogenesis from ependymal radial glial cells and also Müller glia. Zebrafish Tregs have been shown to promote the proliferation of these precursor cells by predominantly producing neurotrophin 3 (NTF3) and NT3 in the spinal cord, as well as insulin-like growth factor 1 (IGF-1) in the retina. So how contrasting and different it is.

Both are done by the same person. It's almost like you have the same coach, but in one place they teach cricket and in another place they teach football. So regenerative factors were upregulated in a tissue-specific manner, which suggests the unique capacity of zebrafish Tregs to produce regeneration factors that are tailored to a specific regenerative context. So Tregs-derived factors, let us look at what the Tregs-derived factors are. One of the major Tregs-derived factors, interleukin 10 (IL-10), is a Tregs-derived immunosuppressive cytokine. It is also anti-inflammatory and promotes tissue repair in mice.

Zebrafish Tregs also express IL-10 in damaged tissue and Tregs-depleted or IL-10 mutant conditions. Keep that in mind. Tregs can produce IL-10, but if IL-10 is mutated, then the Treg is not producing IL-10. Mutant zebrafish exhibited an inflammatory cytokine expression profile because IL-10 is anti-inflammatory. Even in embryos, mouse embryos, if IL-10 is depleted, they lose their regenerative capacity.

So this has been documented. This suggests that IL-10 has a conserved immunomodulatory role in zebrafish Tregs. However, the proliferation of populations of precursor cells such as cardiomyocytes, ependymoradial glial cells, Muller glia, etc., and the expression of regeneration-specific factors such as NRG1, NRF3, and IGF1 were unaffected in the IL-10 mutant zebrafish, which is facilitated and favored by the Tregs. but it demonstrated that the regenerative response mediated by zebrafish Tregs is distinct from the well-characterized IL-10 dependent immunomodulatory function. So just because I have given this example multiple times, if a dish has 15 ingredients and the 16th ingredient is salt, and if salt is missing, those 15 ingredients do not make the food tasty; the same logic should be applied to IL-10.

If it is missing, regeneration can be compromised despite having normal expression of other pro-regenerative factors, so in contrast. The expression levels of the regeneration factor genes were undetectable in Tregs purified from foxp3a mutant zebrafish, which showed that foxp3a is required for zebrafish Tregs to induce the regeneration factors; thus, the expression level of the regeneration factor is not seen at all if Foxp3 is mutated, and Tregs are almost useless from a regeneration point of view. The robust regenerative function of zebrafish Tregs on three distinct precursor populations suggests that Tregs plays an ancestral and universal role in supporting homeostasis and regeneration of diverse tissues by producing growth factors that significantly enhance the regeneration of tissue-specific cell types. This is very important to note that the Tregs plays tissue-specific roles. Mouse Tregs can also modulate tissue regeneration by regulating the proliferation and differentiation of tissue-resident stem cells in specific adult tissues, but it is not powerful enough to bring about a normal regeneration event.

So that also tells you that while Tregs are important, Tregs are not just sufficient to cause a complete regenerative response. However, despite exerting a profound immunomodulatory effect, Tregs are insufficient—indeed, they are insufficient—to promote regeneration inerative tissues, minimally regen such as those of mammals. The heart and central nervous system are weakly regenerating in the mammalian system, but Tregs are not going to supplement that deficiency or complement that deficiency. Interestingly, although the mice lacking Tregs or FOXP3 function succumbed to fatal autoimmune syndrome, zebrafish lacking Tregs function appeared to develop only a moderate inflammatory phenotype; there is no autoimmune syndrome as seen in mammals. This phenotypic discrepancy may be due to the redundancy of FOXP3, as it has 3a and 3b, while the 3a mutant we are discussing in mammals does not have 3b.

Zebrafish have 3b, which is not typically expressed in T cells, but... Because of the absence of 3A, the 3B expression appears in Tregs cells but can be induced in a FOXP3A mutant background through a genetic compensation mechanism. When it is absent, although it is not powerful enough to bring about the regenerative response, it is powerful enough not to have the autoimmune disease as you find in mammals. So it will be very interesting to analyze the immunological phenotypes of mutant zebrafish lacking both FOXP3A and FOXP3B to understand the effects of FOXP3 gene duplication on anti-inflammatory regulation in zebrafish.

Alternatively, the moderate inflammatory phenotype observed in zebrafish lacking Tregs might also suggest that the attenuation of the immune response in aquatic animals, and how it has evolved, will provide good insight into those angles as well. Mammalian Tregs might have lost or have limited pro-regenerative capacity at the expense of gaining a robust immunosuppressive function to harness a rapid and strong inflammatory response that has evolved to seal wounds. There is an injury; forget about regeneration, seal the wounds with impermeable scars in terrestrial animals because they are more vulnerable to wear and tear. There is no water support cushion around their body; they are more vulnerable to dryness and more injury, so a dry environment can favor more of. What you call damage to the body is because there is no cushioning water around it.

So a solid scar is favored. Like zebrafish, neonatal mice can regenerate damaged heart tissue through cardiomyocyte proliferation, but their self-renewal capacity quickly diminishes after birth. One recent study has revealed that the regeneration of neonatal mouse hearts is also facilitated by infiltrating T-reg through the modulation of inflammatory macrophages and the production of growth factors that promote cardiomyocyte proliferation. The factors produced by neonatal Tregs include amphiregulin (Areg), which is required for T-reg-mediated lung and skeletal muscle repair. So the host or the local tissue supports in the form of gene expression favor Tregs

function. Zebrafish Tregs also expresses AREG in a non-tissue-specific regenerative context.

Zebrafish also have the same gene. Thus, the paracrine role of Tregs in cardiomyocyte proliferation is a conserved regenerative mechanism between the zebrafish heart and the neonatal mouse heart. However, it is still important to determine whether the mouse Tregs requires a tissue-specific secretory phenotype in regenerating hearts. It is also important to note that mouse in vivo expansion of T-Rex can reverse autoimmune dysfunction. These studies suggest that genetically modified Tregs could serve as an ideal natural vector for delivering tissue- and disease-specific regeneration factors that activate the latent regenerative pathway. We will study more about regeneration in the upcoming classes. Thank you.