Biomarker and more: can translocator protein 18 kDa predict recovery from brain injury and myocarditis?

“Translocator protein 18 kDa may be more than a simple detector of inflammation in the brain but may be intimately linked with severity and disease pathogenesis. Therefore, our understanding of translocator protein 18 kDa as a biomarker has circled back around to the brain.”

Keywords: biomarker • brain injury • CD11b • complement • imaging • inflammation • myocarditis • sex difference • translocator protein 18 kDa

Translocator protein 18 kDa (TSPO), previously known as the peripheral benzodiazepine receptor, is a well-established biomarker for brain injury and neurodegeneration [1]. The advantage of using TSPO to detect brain inflammation is that it can be measured using noninvasive imaging techniques including PET and single-photon emission computed tomography (SPECT) [2]. TSPO is almost exclusively expressed in inflammatory cells in the brain, and specifically within CD11b⁺ microglia or brain macrophages, making it a very sensitive measure of brain inflammation [2]. Although ligands against TSPO are routinely used to diagnose brain injury, the possibility that the receptor could be driving disease pathology has not been considered by many investigators. Recent findings on the role of TSPO in myocardial inflammation, or myocarditis, are rapidly changing that assessment.

The initial indication that something more was going on came from cardiac microarray gene analysis of mice that had received an injection of coxsackievirus B3 (CVB3), a leading cause of myocarditis in the USA, which indicated that TSPO and its related genes were playing a major role in disease pathogenesis [3,4]. Astoundingly, within hours of infection with CVB3, the spleen, which is basically a sack of immune cells and mainly macrophages, developed a gene profile that was almost identical to what occurs during myocarditis [3]. These findings emphasize that immune cells drive progression to cardiovascular disease. It is known from human myocarditis studies that more

severe or persistent myocardial inflammation predicts progression to arrhythmias, chronic dilated cardiomyopathy and heart failure [5,6]. This led us to examine whether TSPO could be used as a biomarker in the heart to image myocardial inflammation, similar to its role in the brain.

Echocardiography and cardiac MRI (cMRI) are the imaging tools used most frequently to screen for myocarditis [7]. However, neither of these methods as used clinically is able to distinguish inflammation from scar tissue. Instead, echocardiography and cMRI accurately quantify heart function and structure, but changes such as increased wall thickness associated with hypokinesis can only suggest edema or inflammation. A thin, globally dilated ventricle suggests a more chronic stage of myocarditis or a non-inflammatory process such as a titin or dystrophin gene defect. By contrast, cMRI using a combination of T2, early T1 and late T1 is able to identify approximately 80% of recent onset myocarditis cases.

The gold standard for diagnosis of myocarditis remains an endomyocardial biopsy [6]. However, the cost, risks and lack of cardiovascular pathology expertise at most hospitals has limited its widespread use as a diagnostic tool. This means that there is no clinically available, highly specific biomarker or imaging test to identify the presence or severity of myocardial inflammation. Using a mouse model of CVB3-induced myocarditis, we recently determined that myocardial inflammation could be detected using x-ray computed tomography/SPECT (microSPECT

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for mice) directed against TSPO [4]. The next step is to determine whether radioligands directed against TSPO can be used to detect myocardial inflammation in myocarditis patients using PET or SPECT. The availability of this imaging test could transform the diagnosis of myocarditis by enabling doctors to noninvasively identify myocarditis at all stages of disease pathogenesis and, with combined echocardiography and cMRI, possibly predict the likelihood of functional recovery.

“Why not just use a ligand directed against CD11b instead of translocator protein 18 kDa? The advantage of using translocator protein 18 kDa is that it is a ‘promiscuous’ receptor and many different natural ligands bind to it.”

One of the obstacles involved in using TSPO ligands to detect inflammation in the heart is that in contrast to the brain, where TSPO is expressed only in microglia and astrocytes, TSPO is present in the cells of many organs including the heart [8,9]. However, the abundant expression of TSPO in organs also provides a clue to its potential role in disease pathogenesis.

Historically, TSPO has been considered to be a cholesterol-binding receptor necessary for cholesterol transport into the mitochondria for processing to steroids such as estrogens, androgens and vitamin D [1,10]. It is clear that TSPO associates with many genes involved in cholesterol intake and processing such as steroidogenic acute regulatory protein [11]. TSPO levels are highest in tissues where steroid synthesis occurs from cholesterol including the gonads and the heart (the heart produces sex steroids at levels second only to the gonads) [8,9]. This means that the baseline expression level of TSPO is relatively high in the heart, and imaging techniques would need to be sensitive enough to detect invading inflammatory cells. Fortunately, the high sensitivity of current technology makes this possible. Importantly, TSPO is upregulated in immune cells when they become activated, which is part of the reason why TSPO is only expressed in CD11b⁺ immune cells [12]. CD11b is also named complement receptor 3 (CR3) and is upregulated on macrophages, neutrophils, mast cells and some populations of dendritic cells when they encounter antigen. CR3 is the receptor for complement C3, which is the immune system’s natural adjuvant. This means that when CR3 is upregulated, the inflammatory response is greatly amplified. Myocardial inflammation is not necessarily a bad thing – it is needed for destroying pathogens and healing damage to the heart. It is only when inflammatory cells release cytokines and enzymes that initiate cardiac remodeling processes that permanent damage to the heart occurs resulting in fibrosis, dilation and heart failure.

And it turns out that understanding the role of CD11b or CR3 in driving inflammation is the clue to understanding the type of myocardial inflammation that is damaging to the heart. One of the long-standing questions surrounding cardiovascular diseases such as myocarditis, atherosclerosis and heart failure is why they occur more frequently in men than in women [13]. We found that a major difference between myocardial inflammation in males and females with myocarditis is the upregulation of CD11b [14]. We showed that testosterone increases CD11b expression in male mice with myocarditis and testosterone specifically increases TSPO expression in CD11b⁺ immune cells [4,15]. Men with myocarditis express more CD11b and TSPO in their heart compared with women [4]. CD11b⁺ immune cells make up approximately 80% of the inflammation in myocarditis patients, in both men and women. This is particularly important because the cells that express CD11b also express an innate immune receptor called TLR4 and release a cytokine IL-1 β , which is known to promote damaging inflammation, fibrosis and heart failure [16]. We have also found that testosterone increases TLR4 and IL-1 β expression in male mice with CVB3 myocarditis [14,16]. Importantly, it is the expression of CD11b, TLR4 and IL-1 β that predicts whether an individual will progress from myocarditis to dilation and heart failure. Since TSPO is also expressed in these cells, it may be another early biomarker to detect not only the presence of inflammation in the heart, but also to predict whether a patient will progress to dilated cardiomyopathy and heart failure.

Why not just use a ligand directed against CD11b instead of TSPO? The advantage of using TSPO is that it is a ‘promiscuous’ receptor and many different natural ligands bind to it. That means that many different radioligands have been developed and are currently in use to detect TSPO using PET or SPECT [1,17]. These reagents are not available for CD11b. In addition, attempts to activate or inhibit CD11b could result in devastating immune activation or inhibition. Finally, TSPO ligands are already widely used clinically to detect brain inflammation and so are known to be safe to use.

Does the fact that testosterone increases TSPO expression mean that it is only a good biomarker for myocardial inflammation in men? What is important to realize is that the cells of both men and women use testosterone and estrogens as growth factors for normal tissue function; it is just that the ratio of testosterone to estrogen is higher in men. Approximately 80% of the inflammation in female mice with CVB3 myocarditis is CD11b⁺ immune cells that express TSPO; it is just that women usually have far less inflammation than men. This means that TSPO is a biomarker that

should be able to distinguish the severity of myocarditis in patients, suggesting that women with severe cases of myocarditis would express higher levels of CD11b and TSPO. Our data also suggest that TSPO, rather than just being a receptor that provides the fuel for production of sex steroids, is tied to how sex hormones regulate immune function. Mechanistic studies that investigate that relationship still need to be conducted.

What is also exciting about using TSPO as a biomarker of disease severity and progression is that some ligands against TSPO act as agonists while others are antagonists, in a system-specific manner [1]. This means that mechanistic questions can begin to be addressed using these ligands and the recently developed TSPO conditional knockout mice (global deletion of TSPO was embryonically lethal) [11].

Therefore, what does this mean for the brain? Well, interestingly many inflammatory diseases of the brain occur more frequently in men than women including acute ischemia and degenerative demyelination [18].

This suggests that, similar to myocarditis, TSPO may be more than a simple detector of inflammation in the brain but may be intimately linked with severity and disease pathogenesis. Therefore, our understanding of TSPO as a biomarker has circled back around to the brain.

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