REPORT

Possible targeting of G protein coupled receptors to manipulate inflammation in vivo using synthetic and natural ligands

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Cyclic AMP elevating Gs protein coupled receptors were considered for a long time to be immunosuppressive. One of these receptors, adenosine A_{2A} receptor, was implicated in a physiological mechanism that down regulates inflammation and protects tissues from excessive immune mediated damage. Targeting of these receptors by selective agonists may lead to better protocols of anti-inflammatory treatments. At the same time inhibiting the Gs protein coupled mediated signalling with antagonists could be explored in studies of approaches to enhance inflammation and tissue damage. Enhancement of targeted tissue damage is highly desirable when it is cancerous tissue, while enhancement of inflammatory events might be desirable in the development of new vaccine adjuvants.

The need to control inflammation is obvious because prolonged or inappropriate inflammatory responses contribute to the pathogenesis of many major diseases associated with inflammation.[1–5] To harness the body's own natural ability to down regulate inflammation, there is a need to identify endogenous anti-inflammatory pathways. Thus, it is important to identify the molecules that can not only block inflammation but that also play a part in the physiological "sensory" mechanism that recognises when an inflammatory response is damaging rather then beneficial.

One potential candidate among many cyclic AMP elevating Gs protein coupled receptors which may function as the sensory reporter of tissue damage is the A_{2A} adenosine receptor. It has been known for some time that inflammatory tissue damage is accompanied by the accumulation of extracellular adenosine in inflamed areas owing to its release into the extracellular space by a variety of cells (reviewed by Linden[6]); this is a consequence of local tissue hypoxia caused by inflammatory stimuli.[7–9] Moreover, it is now well established that activation of A_{2A} receptors (A_{2A}Rs) on lymphoid cells induced by extracellular adenosine leads to inhibition of an inflammatory response,[10–15] and this is due in large degree to its ability to induce accumulation of intracellular cAMP in activated immune cells. More recently, the use of A_{2A}R deficient mice has illustrated that the A_{2A}R is a critical inflammatory "OFF" button, which is necessary for the inhibition of inflammation and protection from tissue damage.[16] Thus, A_{2A}Rs are promising targets in manipulating the inflammatory response.

Adenosine receptors are members of the family of seven transmembrane G protein coupled receptors. The structure, function, and basis for classification of adenosine receptors and their genes are reviewed by Fredholm *et al*.[17 18] The four known adenosine receptors, A_1, A_{2A}, A_{2B}, and A_3 subtypes, have all been characterised and cloned from a variety of species, including rat, mouse, and human.[7 19–21] Among mammals, the receptors of the same subtype exhibit close similarity.[18] Adenosine receptor distribution is heterogeneous (summarised in Fredholm *et al*[17]). Most data available concern A_1 and A_{2A} receptors because better pharmacological tools are available for detecting those receptors. The A_{2A}R subtype is highly expressed on lymphoid tissues, including the spleen, thymus, leucocytes, and blood platelets.

In general, A_1 and A_3 receptors are coupled to Go and Gi proteins,[22–26] and A_{2A} and A_{2B} receptors interact with Gs proteins.[27 28] In addition, some evidence suggests that adenosine receptors may use other G proteins. For example, A_{2A}Rs may couple with different G proteins in diverse places such as G_{olf} in the striatum,[29] and A_{2B} receptors also link to Gq in some cells.[21 30] By virtue of their interaction with their respective G proteins A_{2A} and A_{2B} activate adenylyl cyclase,[27–29] resulting in the accumulation of cAMP. Activation of A_1 and A_3 receptors inhibits cAMP accumulation.[23 26]

It has been well established that pharmacological activation of A_{2A}Rs on lymphoid cells stimulates an anti-inflammatory response.[11 12 14–16] Investigations into the role of extracellular adenosine have shown that this physiologically abundant molecule triggers strong immunosuppressive responses in T cells, mediated by cAMP, by acting through the Gs protein coupled A_{2A} adenosine receptors (reviewed by Gomez *et al*[31]). In addition, the inhibitory effects of adenosine on the secretion of proinflammatory cytokines by a variety of other cell types, most notably monocytes and macrophages, have been extensively documented.[13 32–38]

In a study using A_{2A}R deficient mice, the first strong evidence for the critical role of A_{2A}Rs in the regulation of inflammation in vivo has been shown.[16] This study demonstrates that absence of the A_{2A}R results in enhanced inflammation and increased tissue damage in models of acute liver damage, endotoxin induced sepsis, and infected wound model. This clearly establishes A_{2A}Rs as negative regulatory receptors of inflammation. Moreover, it illustrates a physiological immunosuppressive loop, in which disturbance of local tissue environment by inflammatory stimuli results in local hypoxia and the accumulation of extracellular adenosine; this, in turn, suppresses inflammation in a negative feedback manner by triggering the accumulation of cAMP through A_{2A}R signalling.

The down regulation of the immune response mediated by A_{2A}Rs is, however, potentially dangerous, despite the beneficial aspects of halting tissue damage and inflammation. The premature inhibition of immune cell function may allow pathogens to survive, and the overall damage to the organism will be greater. On the other hand, the lack of A_{2A}R signalling may lead to excessive damage, with important biological consequences. Thus, the expression and function of A_{2A}Rs should be tightly regulated. Recent studies by Armstrong *et al* demonstrate that there is no receptor reserve in T lymphocytes because $A_{2A}R^{+/-}$ mice had half of the total receptors compared with $A_{2A}R^{+/+}$ mice and only half of the maximal functional

response.[39] Thus, a balance between the need to destroy invading pathogens and the desire to protect tissue from excessive damage ultimately may be dependent on the numbers and level of activation of A_{2A}Rs in individual immune cells.

The identification of A_{2A}R signalling as a physiological pathway for the regulation of inflammation in vivo suggests that targeting of these receptors to manipulate or "engineer" the inflammatory process may have potential therapeutic implications. It is appealing to recruit "natural" anti-inflammatory mechanisms involving cAMP elevating Gs protein coupled receptors to protect tissues from excessive inflammatory damage. Under certain conditions it may be beneficial to augment or prolong selected aspects of an inflammatory process in order to destroy the causative agent, and inhibition of anti-inflammatory pathways may be a therapeutic approach. In principle, the enhancement or inhibition of inflammation might be accomplished through the design and use of more highly selective pharmacological agonists and antagonists. The treatment of a wide range of diseases, including wound healing,[40 41] rheumatoid arthritis,[42] and sepsis,[16] may benefit from targeted triggering of A_{2A}Rs. For example, methotrexate, which is used as the standard treatment for rheumatoid arthritis, has been shown to recruit this pathway by employing the use of endogenous adenosine.[43] Processes that rely on an enhanced inflammation response, such as vaccination, might also benefit from selective targeting. Accordingly, these findings point to the need for more detailed studies of A_{2A}R agonists as recruiters of natural pathways in the treatment of acute and possibly chronic inflammatory processes.

The exacerbation of targeted tissue damage by antagonists of adenosine receptors[16] in models of fulminant and viral hepatitis, as well as in a model of *Pseudomonas* exotoxin A induced liver injury, points to the probable use of antagonists to achieve targeted destruction of tissues and/or targeted enhancement of local immune response in relevant clinical settings. These settings may include the enhancement of anti-tumour immune response or development of better vaccines, or both. These considerations also should include both the synthetic and natural substances that can antagonise adenosine receptors. One such compound is 1, 3, 7-trimethylxanthine (caffeine), which is one of the most widely habitually used psychostimulants. The use of caffeine as a psychostimulant is due to its interference with signalling through adenosine receptors.[17 18] However, substances containing caffeine are also used as treatments in complementary and alternative medicine, including the Gonzalez protocol, in which coffee enemas are an integral part of a proposed regimen for treatment of pancreatic cancer.[44] Thus, recognition of the role of A_{2A} adenosine receptors may provide a rational basis for the analysis of sporadic reports and claims of the therapeutic effects of caffeine.

In summary, the goal of identifying targets for therapeutic modulation can be facilitated by uncovering endogenous mechanisms that stop inflammation. This is greatly facilitated by findings that extracellular adenosine may function as an endogenous "OFF" signal. With this knowledge, it is now possible to engineer (both inhibit and enhance) inflammation by engaging the A_{2A}R mediated pathway.

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