Protein Kinase Inhibitors in Rheumatology

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Abstract: Protein kinases have multiple roles in cell biology, many of which are deeply involved in inflammation and immunity. There are currently many treatment options for patients with autoimmune disease, but none that is completely effective. Protein kinase inhibitors are becoming one of the most promising therapeutic groups, not only for the treatment of disease, but for further developing our understanding of different pathologies. The purpose of this paper is to give an introduction to both protein kinases and their inhibitors, with their implications in the management of autoimmune diseases.

Keywords: Lupus erythematosus, protein kinases, phosphotransferases, Janus Kinase 3, protein kinases inhibitors, rheumatology, autoimmune diseases.

1. INTRODUCTION

Cells respond to environmental changes by perceiving them through extracellular signals. In order to achieve this, a complex network of signal production and reception mechanisms exist [1].

Permeable and non-permeable signals include steroid hormones (in the first group) [2, 3] and growth factors [4], cytokines [5], extracellular matrix components [6], among others, in the later. These are recognized by receptors on the cellular plasmatic membrane [7] that possess a specificity for particular molecules with a similar structure [8].

The binding of a ligand to its membrane receptor has many effects: stimulation of the receptor's intrinsic enzymatic activity or the modulation of a transduction protein (that will ultimately lead to the activation or inhibition of effector proteins [9]). Regulation of activity can be achieved through covalent modifications at a molecular level and, within this possibilities, phosphorylation and dephosphorylation of serine, threonine and tyrosine residues are found [10]. These processes are carried out by kinases and phosphatases respectively [11]. The phosphotransfer reaction requires three specific sites: an ATP binding site, a domain that catalyses the transfer of a phosphate group from the bound ATP, and a substrate binding site [12, 13].

Phosphorylation by protein kinases (PK) is one of the most extended and well-studied signaling mechanisms in eukaryotic cells, being the foundation for cellular signaling networks [14, 15]. It hasn't been an easy task to catalogue and understand protein phosphorylation: Up to 2% of the mammalian genome codifies PK [16], a single cell line can express many kinases and up to one third of intracellular proteins can be phosphorylated [17]. Up until now there are almost 520 identified PK that constitute the "human kinome" [18, 17].

2. DIFFERENT TYPES OF PROTEIN KINASES

Early in the study of PK, Tyrosine kinases (TK) were divided into two groups: receptor tyrosine kinases (RTK) and non-RTK, the last ones are proteins that work "underneath" the RTK in function of signal transduction and amplification of intracellular signals [19, 20, 21]. Receptor tyrosine kinases are trans-membrane proteins that contain an extracellular domain were ligands bind and an intracellular domain with TK activity [22, 19]. Binding of a ligand to the receptor causes stabilization of dimmer, or oligomers, and activation of the tyrosine kinase function of the RTK. This causes an auto-phosphorylation in the tyrosine residues of the intracellular catalytic domain of the activated receptor. The tyrosine residues form binding sites for SH2-domain containing proteins and transmit the inward signal through non-RTK or serine/threonine PK [23]. This classification method was found inadequate and in the 1990's the PK were divided into 5 groups, according to conserved characteristics of their kinase domain [24].

As time has passed, and new technological advances have been made, with the advent of the protein kinase complement for the human genome (the

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E-ISSN: 2310-9874/14

"kinome"), the classification system has been updated. About 518 PK putative genes have been identified and the protein sequences of 56 previously identified PK have been corrected or extended. Thus the previous Hanks and Hunter classification [24] of 5 groups, 44 families and 51 subfamilies was extended by adding 4 new groups, 90 families and 145 subfamilies. This was achieved by comparing catalytic domain sequences, knowledge of similar sequences and domain structures outside the catalytic domain, and known biological functions [16].

3. PROTEIN KINASE STRUCTURES

The PK family shares a catalytic domain with a conserved sequence and structure but with a great difference in how its function is regulated. The ATP binding site is located between the 2 lobules of the PK: an N-terminal sub-domain that consists mainly of Beta sheets and a C-terminal sub-domain made up predominantly by Alpha helix [25]. The two sub-domains can rotate into "open" and "closed" conformations depending on ATP binding and the molecule's activation state [26]. The highly conserved catalytic domain is located on the external border of the ATP binding site, and it is the main target of PK inhibitors (PKI) that exploit structural and flexibility differences to achieve greater selectivity [27]. Before the catalytic domain can accomplish its phosphotransfer function, both the ATP and the substrate protein must be at their binding sites, making them plausible targets for the development of function inhibitors [28] (Figure **1**).

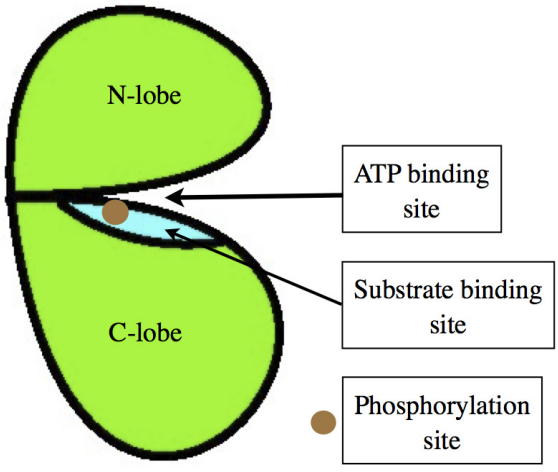

Figure 1: Protein kinase basic structure.

Right now PK are amongst the major groups of pharmacological targets [29]. Disadvantages arising from targeting the ATP binding site are competition between the compound and intracellular ATP, and the necessity for telling apart ATP binding sites of different PK from the ones of other human proteins that also use ATP. Because of this two reasons the possibility that PK could become adequate therapeutic targets with effective inhibitors was considered almost impossible [29]. Evidence of how a drug that was already in clinical use acted by modifying the phosphorylation state of one or more intracellular compounds was put forward [30] when the intracellular targets of cyclosporine and FK506 (tacrolimus) were discovered [31], and with Rapamycin's at the end of the same decade [32]. Besides, there is now enough evidence of some variability between ATP binding sites of the different PK as to allow sufficient selectivity [33] and that access to active sites can change between protein kinases.

4. PROTEIN KINASES AND DISEASE

Signal transduction routes have important roles in the control of adequate cellular functioning, the state of a signaling protein kinase determines cellular state, and its alterations result in abnormal signal transmission [34, 35].

Anomalies in signal transmission can have an impact in many and various cellular processes (Table **1**): angiogenesis, apoptosis and cellular migration, control of cellular cycle and the development of malign phenotypes [36, 37, 38, 39]. They have also been involved in the genesis of autoimmune pathologies [40] and, as a consequence, they have become an important therapeutic target for this diseases [41, 42, 43].

Table 1: Mechanisms that Can Modify PK Activity

Mechanism	Consequence
Genetic rearrangement with generation of a hybrid protein	Bcr-Abl mutation that causes chronic myeloid leukemia is an example [44]
Mutations that cause a constitutively active kinase [45].	Resistance to tyrosine kinase inhibitors
Deregulation of kinase activity by activation of oncogenes or loss of suppressor genes [8].	Different pathways implicated in cancer pathogenesis [8].
Augmented or aberrant expression of PK or the RTK's ligand [39].	Acute myeloid leukemia

5. Protein Kinases and Inflammation

PK have various roles in the inflammatory cascade both as promoters and regulators, which can be seen

on their effects over the production of interleukin 1 (IL1) [46, 47] and tumor necrosis factor (TNF) production [46].

In the classical innate inflammation pathway, before PK can be activated, high affinity receptors must recognize molecular patterns and antigens. After recognition of these signals, molecules are recruited towards the cytosolic region of the receptors and activate oligomerization of PK proximal to the IRAK family receptor (IRAKI-4) [47] and MAPK (Mitogen activated protein kinase) kinase kinase (MAP3K) [48]. By means of ubiquitination, signals of proteins like IκBα (Nuclear Factor–kappa-B inhibitor alpha) [49] or TNF receptor associated Factor 3 [50] are halted by being targeted for proteasome degradation (Figure **2**).

The activated MAPK phosphorylate nuclear proteins taking part in the induction of genetic transcription by NF-κB [51], through recruitment regulation of the NF-κB and histone modifications [52], simultaneously activating proteins that stabilize messenger RNA and liberating translational blockade [53] (Figure **2**).

Other protein kinases are involved in the regulation of pro-inflammatory cytokines production, as is the case of Janus kinases (JAK) and their role in the development and cytokine production profile of dendritic cells [54]. They also participate in modulating the response to cytokines and interferon, like the TAM family RTK, “recepteur d'origine nantais” (RON) RTK, lymphocyte specific TK (LCK) and spleen tyrosine kinase (SyK) [55].

Other PK, like ZAP70 and Bruton’s tyrosine kinase (BTK) have a direct relation with activation of p38α in an MAPK kinase dependent and independent manner. They are central components of inflammation’s classical signaling pathways [36] (Figure **2**).

6. PROTEIN KINASE INHIBITORS

Most of the PKI bind to the ATP pocket in the small lobule of the PK. Their mode of action varies from orthosteric [56] and competitive inhibition of ATP [57] to allosteric inhibition mechanisms [58]. They can also bind the PK and “enclose” them in an inactive conformation, and they can extend their targeting to residues close to the ATP binding site [27, 59].

Traditionally, protein kinase inhibitor discovery has been made in a lineal manner [60], where selectivity of

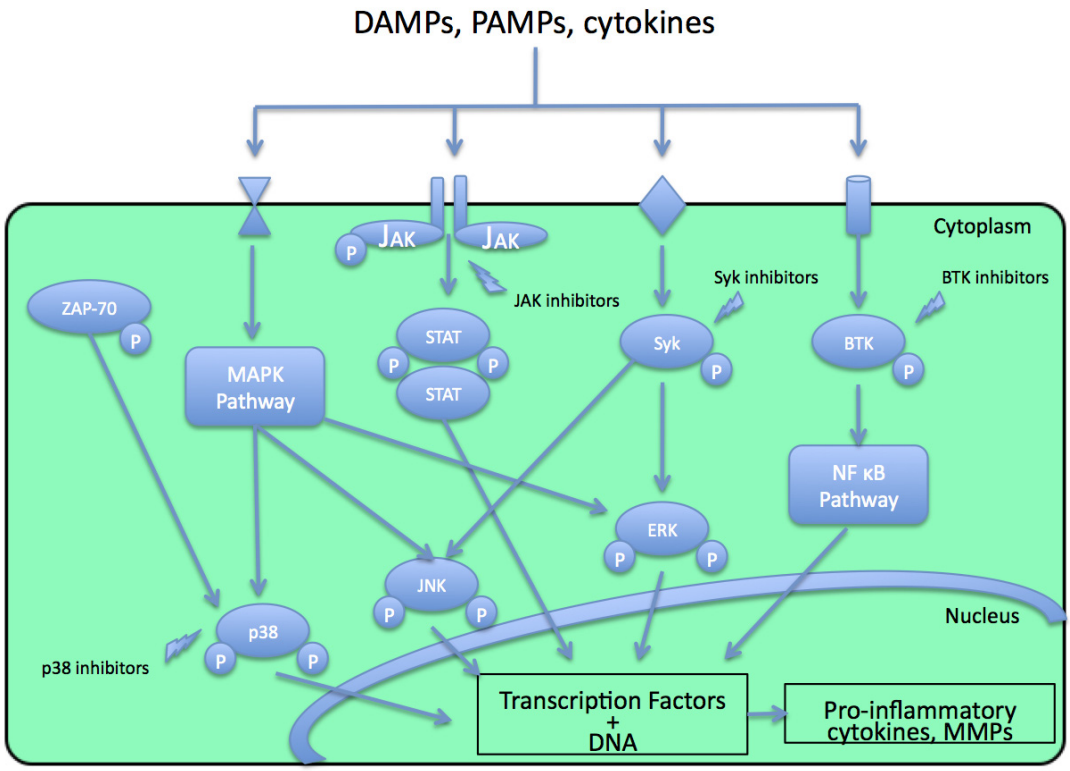

Figure 2: DAMPs, danger associated molecular patterns; PAMPs, pathogen associated molecular patterns; JAK, Janus Kinase; MAPK, mitogen activated protein kinase; Syk, spleen tyrosine kinase; BTK, Bruton’s tyrosine kinase; JNK, c-Jun N-terminal kinase; ERK, extracellular signal-regulated kinase; MMPs, metalloproteases; STAT, Signal transducer and activator of transcription; NF-kB, nuclear factor kappa B.

the inhibitor is evaluated only in a small group of chosen compounds and is checked upon in a sporadic manner throughout the entire process.

Only one compound is evaluated at a given time (repeating the whole process each time a compound of interest is found), and there isn't much thought given to availability and quality of the investigated compound.

Multiple alternate screening strategies have been developed in search of efficient discovery and development methods. Examples of these are: screening based on ATP binding site crystallized structures [61], of members of the same family or of already known compounds with ATP pocket binding capabilities (a technically difficult way because of the highly phosphorylated state of the protein) [62], high output techniques to identify kinase profiles [60], development of several kinase panels and their inhibitors, and rational drug design [63].

The requirement that the PKI have a high affinity, and many orders greater, than the one that the PK has for ATP is another obstacle because of the lower concentrations the PKI will reach within the cell.

Selectivity filters are the result of rational drug design and have been used to augment inhibitor specificity. An example of this method is the development of PKI for ribosomal S6 p90 kinases (RSK): Targeting two determinants in the ATP binding pocket the specificity of the compound is enhanced [64]. Compounds that inhibit in a highly selective manner closely related kinases have been made available with a theoretical reduction in the risk of resistance generating mutations [63, 64] by having the inhibitor target two sites.

Modulation *via* small peptides is another option for kinase inhibitors with higher specificity. This compounds can be specific either for a particular kinase or a protein-to-protein interaction [65], they don't cause an important alteration in the endogenous protein balance and effectiveness has been shown at low nanomolar concentrations *in vivo* [66].

The development of several techniques to "carry" peptides into the cell, with *in vitro* and *in vivo* models, has been advantageous [67]. One of the disadvantages of the small peptides is their high turnover rate (which depends on their composition), but it is also one of their strong points, because it could lead to fewer side effects. Within the different types of small peptides, we can find: pseudo-substrate peptides, binding site peptides and protein-to-protein interaction modulators [67].

Maximum selectivity might not always be the best choice. An example of this is the clinical use of Imatinib which was initially thought as highly specific for its main target but, as clinical experience was gathered and with a better knowledge of the molecule, it was found that this drug was not completely selective (until this day none of the approved PKI are!). It also had the ability to inhibit both KIT and platelet derived growth factor (PDGFR), making it useful in the management of gastrointestinal tumors and eosinophilic syndromes, expanding its clinical utility [68].

7. PROTEIN KINASE INHIBITORS IN RHEUMATOLOGY

Acute inflammation is typically regulated after its stimulus has been removed but, in diseases like rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), there is a chronically maintained inflammatory state that leads to harmful local and systemic effects [69].

A high number of therapeutic options have emerged for chronically inflammatory states: steroidal anti-inflammatory and non-steroidal anti-inflammatory drugs, disease modifying slow acting drugs, immune-suppressors and biologicals with inflammatory cytokines as targets (TNF, IL1) have been used [55].

All of the options have significant disadvantages: various adverse effects and partial responses. Regarding biologics, high production costs with complex manufacture, intravenous or subcutaneous administration, incomplete response in up to two third of patients and recurrence once treatment has stopped, have made the development of new molecules that control chronic inflammation a necessity [55].

Protein kinases represent attractive targets for modulating pro-inflammatory reactions in different cell types in a simultaneous and receptor specific independent manner [70, 29], given the fact that cells involved in inflammatory reactions utilize highly conserved signaling networks for their control and for modifying genetic responses involved in these actions.

7.1. Mitogen Activated Protein Kinases (MAPK)

Inflammatory stimuli activate four mayor intracellular signaling pathways: NF-κB and three MAPK ways. The MAPK signaling pathways are p38, ERK (extracellular

signal regulated kinase) and JNK (N-terminal c-Jun). These networks compromise the sequential activation of multiples kinases: MAPK are activated by MAPK kinases (MKK), which are activated by MAPKK kinases (MKKK). So, p38 (isoforms α, β, γ y δ) are activated by MKK3 and MKK6; ERK (both 1 and 2) are activated by MAPK-ERK kinases (MEK) 1 and 2, and JNK (1, 2 and 3) by MKK4 and MKK7 [53, 46].

7.1.1. p38

The initial enthusiasm for p38 inhibitors, mainly in RA, has been withering. The first generation of this compounds, whose targets were the four isoforms of p38, failed to achieve their objectives in clinical trials due to hepatic, central nervous system and skin toxicities [71, 72]. When the importance of p38α in RA was discovered, by promoting pro-inflammatory cytokine expression and osteoclast formation [73], new enthusiasm was given to this molecules. Unfortunately, this highly selective compounds didn't fare any better that their non-selective predecessors: hepatic enzyme elevation (VX-745 and BIRB 796) [74, 75]; little efficacy (SCIO-469, Pamapimod) [76, 77] and skin toxicity (SCIO-323) [78] have been some of the explanations why this drug class hasn't advanced any further in clinical trials.

An explanation to their adverse effect profile can be made through the recently identified anti-inflammatory function of p38α [55], explaining why its inhibition would lead to a state of non-controlled inflammation and the already mentioned side effects.

In other disease, like SLE, there hasn't been as much research as in RA, although the importance of PK in their pathogenesis is known. Until the moment of this review, there aren't any PKI in human clinical studies of SLE. They have been tried in animal models showing a positive impact on the disease's renal affectation [79].

Still, molecules either "upstream" or "downstream" p38 can become interesting targets for inhibition. "Up" from p38 are MKK3 and MKK6, avoiding adverse effects on the defense mechanisms of the individual [55]. "Downstream" p38, like MK2 and MK3, leaving control pathways and anti-inflammatory molecules intact [55].

7.1.2. MEK-ERK

MEK-ERK pathways are involved in cellular proliferation and pro-inflammatory processes [75]. Signaling of MEK-ERK is augmented in RA synovium, and it induces the production of pro-inflammatory cytokines, modulates signaling from both the B cell receptor (BCR) and T cell receptor (TCR), among others [80, 81, 82].

MEK 1 and 2 inhibitors have been developed and have shown efficacy in RA animal models with suppression of synovitis, pannus formation and bone erosions [80, 81, 83]. Unfortunately in human clinical trials an MEK1/2 inhibitor (ARRY-162) didn't show to be any better than placebo in refractory disease [84]. In what respects to MEK-ERK inhibitors, there has been proof of efficacy in murine models, but the fear of inducing some type of lupus-like disease has raised doubts about their safety [85].

7.2. JNK

Both stress and cytokine signals activate the 3 isoforms of JNK (JNK 1, 2 and 3) which have important roles in apoptosis, inflammation and extracellular matrix degradation [86]. Both JNK 1 and 2 have a general expression in different tissues and their phosphorylation is detected in RA synovium [75].

Activation of JNK by SyK leads to the production of interleukin 6 (IL6) and matrix metalloproteinase (MMP) (critical in joint destruction) [75] and differentiation of T cells to Th1 [87]. Blockade of JNK must be simultaneous to be effective, as has been suggested in animal models [75]. The JNK pan-inhibitors SP600125 and AS601245 have shown improvement in inflammation and damage of joint cartilage and bones in murine models. The problem with this compounds is that they aren't selective enough, and this could lead to, yet unknown, side effects [84]. The answer to this lack of specificity could lie in the development of JNK inhibitors based on protein-to-protein interactions, some of which now exist, but the fear of tumor producing side effects (because of the JNK role in apoptosis) is a significant concern [88].

7.3. Tyrosine Kinases (TK)

The RTK family includes insulin receptors and several growth factor receptors, like endothelial growth factor (EGF), fibroblast growth factor (FGF), PDGF, vascular endothelial growth factor (VEGF) [23]. In the non-RTK family, we can find Src, JAK, Abl, among others [19].

TK are integrated into inflammation in two manners: they are an important components of the signaling pathways initiated by toll like receptors (TLR) (being

critical for cytokine production) and many cytokines (including TNF, IL6 and IL10) also use TK in their own signaling networks [89].

7.3.1. Janus kinases (JAK)

There are 4 identified JAK (JAK 1, JAK 2, JAK 3 and TyK 2) [90] with an extremely important role in both innate and adaptive immunity, participating in the transmission of signals from cytokine receptors that lack intrinsic kinase activity. Both JAK 1 and 3 are responsible for the transduction of signals from the common γ chain of the IL 2, IL 4, IL 7, IL 9, IL 15 and IL 21 receptors (critical for activation, function and proliferation of lymphocytes) [91, 92]. JAK1 can combine with other members of the JAK family and signal for other cytokines, for example, JAK 1 and 2 for IL6 and γ interferon, JAK1 and TYK2 for α/β interferon [93]. JAK 2 is expressed in a constitutive way and is essential for hematopoiesis [94].

JAKs are recruited after the ligand binds to its receptor; they bind to the cytoplasmic tail of the receptor complex and go through auto-phosphorylation. The phosphorylated JAK promote the binding of STAT proteins (transcription activators and signal transducing proteins), which are phosphorylated on their tyrosine residue at the C-terminal position [92]. Phosphorylated STATs dissociate from the JAK complexes and dimerize in the cytoplasm, they translocate to the nucleus and associate with gene promoter regions to mediate transcription and regulate the individuals immune response [93, 95]. After transcription is completed, STATs are de-phosphorylated and translocate back to the cytoplasm [96].

Because of their roles in innate and adaptive immunity, it is logical to assume that JAK are involved in RA pathogenesis. Inhibition of JAK3 ameliorates clinical manifestations and protects against joint damage in RA animal models [97]. There have also been found anti-inflammatory effects on transplantation and pulmonary inflammation induced by allergens [98, 99].

The first “selective” inhibitor tried in humans was tofacitinib (previously tasocitinib or CP-690,550). It inhibits in a potent way both JAK3 and JAK1, with little effect on JAK2 and TyK2, and with a selectivity about 1000 times greater for JAK than for any other type of kinases [95, 100]. In regard to its action mechanism, it blocks common γ chain cytokine action (IL2, IL4, IL15 and IL21), inhibiting differentiation of T cells to a Th2 subtype. Also, by inhibiting signaling of γ interferon, IL12 and IL3, the differentiation to Th1 and the generation of Th17 are blocked. It also limits the production of TNF in murine models and blocks the effects of IL6 and type I interferon on synovial fibroblasts [93, 95, 91].

In human studies, Tofacitinib has caused clinical improvement [101] as monotherapy in patients with inadequate response to at least on non-biological or biological disease-modifying drug, clinical and radiological improvement in patients with poor response to methotrexate [102] and clinical benefit in combination with methotrexate in patients refractory to a TNF inhibitor [103]. Apparently it is also similar to adalimumab in methotrexate refractory patients [104].

Tofacitinib’s side effects appear to be mostly related to its mechanism of action. They are mainly an increase in respiratory infection, M*ycobacterium tuberculosis* infections, anemia and neutropenia, elevation of low density lipoprotein and transaminases [101]. It is interesting that, though it has shown a positive profile on the joint histology in animal models, it has been found that JAK inhibitors can increase nuclear levels of NF-ATc1 and cJun with an induction of osteoclast-like cells and augmentation of areas of resorption bone cell culture media [105].

There are other JAK inhibitors that are currently under investigation for both immune and hematological diseases. Inhibiting JAK 1 and 2, Ruxolitinib has shown efficacy in a small randomized human study of RA and animal models [105, 106]. Still, the current FDA approval is for myelofibrosis, and it is in hematological disorders where most of the literature about de compound is found [107]. Other JAK inhibitors that have had some study in autoimmune diseases are LY3009104, previously INCB028050 [100], with some improvement in patients refractory to DMARD and biologics [108].

There are few molecules that have had studies in SLE: CEP-33779 is a selective JAK2 inhibitor that has had some positive results in SLE animal models [109]. It has been shown to deplete auto-reactive plasmatic cells and cause improvement in an animal model of nephritis [110]. AG-490 inhibits phosphorylation of JAK2 and STAT1: in murine MRL/lpr models it diminishes proteinuria, improved renal function, suppressed renal and salivary gland histology lesions, it decreases the anti-dsDNA concentration and the IgG and C3 deposits in the renal parenchyma while decreasing serum concentrations of monocyte chemotactic protein-1 (MCP1) and γ interpheron [111].

7.3.2. SyK

SyK is expressed in all of the hematopoietic cells, it mediates immune-receptor signaling (like the ones of BCR or the FcγR) [112, 113]. It is also found in non-hematopoietic cells, where it handles the transduction of signals from TNF, IL1 and LPS [21].

The binding of its ligand to the SyK associated receptor leads to the phosphorylation of intra-cytoplasmic ITAM (immune receptor tyrosine activation motifs) by Src family kinases. This leads to the recruitment of SyK and its association with ITAM through SH2-domains. This causes SyK activation, which leads to phosphorylation of adaptive proteins, causing gene activation and cytoskeleton rearrangement [21, 114, 115].

In the signaling pathway through FcεRIγ (gamma chain for the high affinity IgE receptor), SyK is involved in the activation of multiple functions such as phagocytosis (macrophages), bone resorption (osteoclasts) or cytokine production (macrophages and T cells) [116]. All of this has led SyK to become one of the principal therapeutic targets for inflammation and rheumatic diseases.

SyK's activity is increased in the synovium in RA [117], where it regulates the production of IL6 and MMP3 in TNF stimulated fibroblast-like synoviocites [118], and it also promotes osteoclast activity [41].

In SLE, there is a re-arrangement of the signaling pathways of the TCR, using a FcRγ and SyK instead of the usually used CD3ζ and ZAP70. There is less production of IL2 but with higher levels of IL17 because of an increase in intracellular calcium with a malfunction of the rest of the branches in this signaling pathway. The result is a T cell population that has lower activation thresholds and a more potent response to activating signals [35].

There have been many compounds developed, the most successful one until now is fostamatinib (R788), which is a pro-drug of the SyK inhibitor R406. The last one has limited selectivity because it has been found to have the ability to inhibit both kinase and non-kinase proteins (JAK 1 and 3, and FLT3) [115].

In RA animal models, both R788 and R406 showed a reduction in both inflammation and bone erosions [115, 119]. In human synoviocyte cell cultures, a potent anti-inflammatory activity was shown [117]. In humans with RA they have demonstrated an improvement in symptoms, inflammation markers and visualization of synovitis with magnetic resonance of the joints, but clinical improvement has not been equally distributed in the different groups [120, 121]. Most common side effects were nausea, diarrhea, neutropenia and transaminase elevation.

In SLE, up until now, there is only evidence in murine models. They have shown a delay in proteinuria appearance, renal dysfunction and an increase in survival for NZB/NZW mice; and a reduction in severity of skin and renal manifestations in both MRL/lpr and BAX/BAK models, without modifying the levels of anti-DNA antibodies [122, 123]. All of this could suggest that inhibition of SyK in humans suffering of SLE could have great clinical significance.

Due to lack luster results AstraZeneca announced in 2013 the decision not to proceed with regulatory filings for RA with the FDA and returned the rights of the compound to Rigel Pharmaceuticals [124]. Currently there are plans to continue the study of fostamatinib in patients with idiopathic thrombocytopenic purpura by Rigel Pharmaceuticals [125].

Imatinib, a PKI for Bcr-Abl, c-KIT and PDGFR, which has been used mainly in malignancy, has shown improvement of symptoms and inflammation markers in animal [126] and human reports of subjects with arthritis [127]. This is explained, in part, by its capacity to inhibit SyK [128]. Its implementation in clinical practice for arthritis has been limited because of the reports of important side effects. The lack of publication of a clinical trial conducted by Novartis in patients with RA causes doubts on its utility and safety [129]. This molecule has also entered the field of diseases that cause fibrosis (like systemic sclerosis and pulmonary fibrosis), because of its ability to inhibit [130] and decrease the production of TGF β [131]. Still, available data has not shown a clear advantage on its use with the shortcoming of side effects on up to 30% of patients [42, 132].

8. OTHER KINASES

8.1. Rho Kinase

Rho kinases (ROCK) are serine/threonine PK that handle the transduction of signals of the Rho proteins responsible of regulating various cellular aspects like shape, motility, proliferation and apoptosis. Rho Kinases have two isoforms, ROCK1 (ROKβ or

p160ROCK) and ROCK2 (ROCKα). Both isoforms share a 65% of their amino acid sequence and a 92% of their kinase domains [133]. It has been observed that inhibition of ROCK decreases cytoskeleton anomalies in T cells from lupus patients [134], production of IL2 and γ interferon [135], IL17 and 21 [136].

Administration of Fasudil (a potent ROCK inhibitor and vasodilator) to MRL/lpr mice caused a reduction in the production of IL17 and 21 in spleen, of sIgG1+ B cells and plasmatic cells with a marked reduction of anti-dsDNA antibodies, IgG and C3 glomerular deposits and proteinuria [136]. In a similar way, in NZB/WF1 mice, an increase in survival, a decrease in proteinuria, anti-dsDNA, IgG and C3 deposits, glomerulonephritis, effector/memory T CD4+ cells and of plasmatic cells has been found [137].

These compounds have low selectivity and the possibility of important side effects [133]. Fasudil is already licensed in Japan and China for the management of cardiovascular problems.

8.2. Bruton's Tyrosine Kinase (Btk)

The Tec family (tyrosine kinases expressed in hepatocellular carcinoma) of non- RTK is the second largest one, after SFK. Btk is a member of this family, and it is expressed mainly in B cells, myeloid cells and platelets [138, 139, 140]. It is responsible for LPS signaling mediated by TLR through two pathways: NFκB and p38 [36]. It causes an increase in the production of TNF and IL1 [21], transduces signals from BCR and FcεR1 [141] and is directly involved in the adequate development of B cells (as shown by X linked agammaglobulinemia) [142].

All of the above make Btk an interesting target for the control of different autoimmune diseases. The inhibitor PCI-32765 is in development for B cell non-Hodgkin lymphoma, and it has also been studied in autoimmunity models by researching the effects of Btk inhibition on mature B cell function. This compound inhibits signal transduction from the BCR without any effect on the TCR [143]. It has also shown suppression of auto-antibody production and improvement in manifestations of induced arthritis models [144], and, in MRL/lpr mice the inhibition of auto-antibody production and development of renal compromise [143].

The compound GDC-0834 has shown clinical improvement of collagen induced arthritis in rats, and also at histology level [145].

A different approach from the "traditional" one has been seen with the META060 molecule. META060 inhibits the activity of Btk, SyK, phosphatidyl inositol 3 kinase (PI3-K) and glycogen synthase kinase 3β (GSK3β), breaking the traditional scheme of "maximum selectivity". *In vitro* RA models demonstrate a reduction in phosphorylation of β catenine, TRAP activity and inhibition of the activation of E2 prostaglandin, MMP3, IL6 and IL8 activated by IL1β. In mice with acute inflammation, it reduced paw inflammation in a similar manner that aspirin did. In mice with collagen induced arthritis it also decreased clinical arthritis indexes and joint cartilage and bone degradation, it also lowers IL6 levels [146].

Other compounds that inhibit Btk like compound 4 and CGI1746 have shown some efficacy in collagen antibody induced arthritis and collagen induced arthritis models, respectively. Even dasatinib has been used in animal models, showing certain improvement [147].

8.3. C-Fms (Colony Stimulating Factor Receptor 1)

C-Fms is a key factor for regulating cells of the monocytic lineage. Its ligand, macrophage colony stimulating factor (M-CSF), is produced in fibroblast-like synoviocytes, T cells and endothelial cells, with an over-expression in RA [148] and its animal models [149]. In the later, its inhibition has shown improvement in clinical scores, histology, pannus formation, bone erosion and cartilage damage [150]. These are the reasons why a phase I study of the molecule PLX5622 is on its way, in patients with RA that are taking methotrexate [151]. Its effects could be really interesting as monocytes and their derivates are the main targets.

8.4. PKCθ

PK C theta, of the serine/threonine kinase family, is expressed in T cells, muscle-skeleton cells and platelets. During T cell activation, this PK carries signals from the TCR to effector molecules NF-κB, AP-1 and NFAT. This results in secretion of IL2, increased IL2 receptor expression and the clonal expansion of T CD4+ and CD8+ cells [152]. In antigen induced arthritis models, mice deficient for PKC theta developed the disease but in less severe form than the wild type did, with less cellular infiltration and dame to joint cartilage; in the collagen induced arthritis model the clinical score for damage was lower, and there was protection from bone destruction. These PKC theta deficient mice had also an alteration in the proliferative response of T

CD4+ cells, lower γ interferon, IL2 and IL4 at intracellular level; it also decreased markers of memory T cell activation [153]. Because of these findings mentioned above, the next logical step would be the development of selective inhibitors for this protein kinase taking into account the important role it plays in cell mediated immunity [154].

8.5. mTOR (Mammalian Target of Rapamycin)

The target of rapamycin (TOR) is a conserved serine/threonine kinase that regulates cell growth and metabolism in response to environmental cues. TOR is part of two complexes: TOR complex 1 (TORC1), which is sensitive to rapamycin, and TORC2, which is not. The consequences of mammalian TORC1 alteration suggest that inhibitors of mTOR could be useful for the treatment of multiples diseases, rheumatological conditions included [155]. mTOR signaling is increased in SLE T cells, and inhibition of mTOR signaling with rapamycin has been shown to lower baseline calcium levels and reduce calcium influx following TCR stimulation. It also promotes regulatory T cell, tolerogenic dendritic cell expansion and limits pro-inflammatory IFN alpha production [156]. In murine models, it has improved survival, decreased albuminuria and damage in affected organs in MRL/lpr mice [157]. In NZB/W F1 lupus prone mouse, it lowered anti-dsDNA titers and deposition of IgG and C3 in the kidney [158-160], and a small group of human SLE patients with refractory disease had a reduction in steroid dose and burden of disease [161]. There is currently a phase 2 study in development aiming to determine the therapeutic effect and mechanism of action of rapamycin in patients with SLE [162].

Table 2: Effects of PKI's

Targeted PK	Effect
p38 non-selective	Hepatic, central nervous system and skin toxicities [71, 72].
p38α	Hepatic enzyme elevation (VX-745 and BIRB 796) [74, 75] Limited effectiveness (SCIO-469, Pamapimod) [76, 77] Skin toxicity (SCIO-323) [78]
MEK 1/2	RA animal models: suppression of synovitis, pannus formation and bone erosions [80, 81, 83] ARRY-162: didn't show to be any better than placebo in human refractory RA [84] Fear of inducing lupus like disease [85].
JNK	SP600125 and AS601245: improvement in inflammation and damage of joint cartilage and bones in murine models [75]. Unknown, side effects [84].
JAK	Tofacitinib (JAK3 and 1): clinical and [101] radiological [102, 103] improvement in patients with RA. Increases risk of opportunistic infections, elevation of hepatic enzymes and neutropenia [101]. Ruxolitinib (JAK1 and 2): benefit in randomized human study of RA and animal models [105, 106]. LY3009104: Some improvement in patients refractory to DMARD and biologics [108]. CEP-33779 (JAK2): In SLE animal models [109] depletes auto-reactive plasmatic cells and causes improvement in an animal model of nephritis [110]. AG-490 (JAK2): In murine MRL/lpr models diminishes proteinuria, improves renal function, suppresses renal and salivary gland histology lesions, decreases anti-dsDNA concentration and IgG and C3 deposits in renal parenchyma [111].
SyK	Fostamatinib: In RA animal models reduction in inflammation and bone erosions[115, 119]. In humans with RA improvement in symptoms, inflammation markers and visualization of synovitis with magnetic resonance of the joints [120, 121]. Most common side effects were nausea, diarrhea, neutropenia and transaminase elevation. In SLE, in murine models, delay in proteinuria appearance, renal dysfunction and an increase in survival, reduction in severity of skin and renal manifestations [122, 123]. Application for FDA approval in RA not pursued.
Rho Kinase	Fasudil: In MRL/lpr mouse caused a decrease in the production of IL17, IL21, sIgG1+ B cells and plasmatic cells with a marked decrease of anti-dsDNA antibodies, IgG and C3 glomerular deposits and of proteinuria [136]. In NZB/WF1 mouse an increase in survival and a decrease in proteinuria, anti-dsDNA, IgG and C3 deposits, glomerulonephritis, effector/memory T CD4+ cells and of plasmatic cells [137].
Btk	PCI-32765: Suppression of auto-antibody production and improvement in manifestations of induced arthritis models [144]. In MRL/lpr mice the inhibition of auto-antibody production and development of renal compromise [143]
C-Fms	PLX5622: improvement in clinical scores, histology, pannus formation, bone erosion and cartilage damage [150]
mTOR	Rapamycin: Improved survival, decreased albuminuria and damage in affected organs [157], lower anti-dsDNA titers and deposition of IgG and C3 in the kidney [158-160], in SLE mouse models. In a small group of human SLE patients, it led to a reduction in steroid dose and burden of disease [161]. Phase 2 study in development [162].

MEK: MAP kinase or ERK kinase; JNK: N-terminal c-Jun Kinase; JAK: Janus Kinase; SyK: Spleen tyrosine kinase; Btk: Bruton's tyrosine kinase; C-Fms: colony stimulating factor receptor 1; mTOR: mammalian target of rapamycin.

9. CONCLUSIONS

Autoimmune diseases are a complex field with great advances in the last decades thanks to the development of new drugs in the quest for better therapeutic options. Because of the shortcomings of the current available medications, their costs and complex manufacture (the biologics above all), their side effects and their complicated administration ways (subcutaneous or intravenous); the development of newer molecules has become a necessity for satisfying the demands of the population that is living longer, and that has survival rates much higher than in past times, which has led to the appearance of complications and therapeutic failures that didn't exist in other eras.

Within the new molecules that have appeared, the protein kinase inhibitors are still a giant field to be explored and from which much can still be learned. At this moment, it appears there is a tradeoff between the greater selectivity that has been achieved with some of the new medications in rheumatology (anti CD20, anti BAFF, among other) for compounds that, though try to be "selective", still haven't achieved the single target specificity. When this quality is lost, that is when undesirable side effects that have led to abandon entire compound families, appear (Table **2**).

Advances in the understanding of the pathogenesis of SLE and RA have been the main force behind the development of PKI, and it is exciting to see how all of the recently discovered information is exploited to have a positive influence in the diseases. Although practical applications on RA are the ones leading the way, we have no doubt there is little time before clinical trials in lupus are initiated, based on the encouraging results with protein kinase inhibitors in animal models of this disease.

If the answer is "ultra" selective molecules or, by the contrary, it is in "pan" inhibitors with variable selectivity for their possible targets, it's still too early to know. What can clearly be anticipated is that the addition of these drugs to the therapeutic arsenal, a reality in RA with the FDA approval of tofacitinib in November 2012, will give us one more option in those complex patients with important side effects and inadequate control of their disease for whom the current medications aren't enough.

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Received on 10-02-2014 Accepted on 04-03-2014 Published on 26-03-2014

DOI: http://dx.doi.org/10.12970/2310-9874.2014.02.01.4