

## REVIEWS

# Mechanism-based pharmacodynamic interactions of glucocorticoids and disease-modifying antirheumatic drugs: A review

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### Abstract

Combination of conventional disease-modifying antirheumatic drugs (cDMARDs), which assist in achieving effective disease control and functional outcomes in rheumatoid arthritis (RA), is widely accepted as an effective treatment regimen. Steroid is often used in combination with DMARDs and there are several uncertainties associated with the regimen, including the therapeutic benefits of the combination. Further understanding the pharmacodynamics and mechanism of actions of steroids and possible drug interactions may assist in improving the treatment outcomes. While the treatment has shown to be effective with better outcome, the use of steroids in the combination regime has been a topic of contradiction.

The pharmacodynamic actions of corticosteroids and cDMARDs is variable and overlapping in some domains. In a highly dynamic and constantly adopting immune system, the expression of receptors and signal pathways depends on various factors including the status of cytokine and antigen load. The pattern of introduction of the drugs is one of the major challenges that hampers the effective use of synergism and other interactions associated with the combination. Understanding the drug mechanism and interaction pathways and their variability is essential for customizing the treatment strategies. This review article addresses the current trends of combined treatment in the management of RA. It is also aimed at providing consolidated information on the pharmacodynamic interactions of glucocorticoids and DMARDs in RA.

**Keywords:** Pharmacodynamics, RA, DMARDs, glucocorticoids, NF- $\kappa$ B, T cells

### Introduction

The management of rheumatoid arthritis (RA) has witnessed a gradual shift towards the use of combined disease-modifying antirheumatic drugs (DMARD). There are trials demonstrating the benefits of combining the steroid along with DMARDs. However, there are observations indicating increased mortality in RA patients treated with steroids in combination with DMARD.<sup>1-3</sup> Higher glucocorticoid dose was associated with all-cause and cardiovascular mortality in RA. But certain researchers believe that the increased mortality could be due to the bias in selecting patients to receive steroids. A recent analysis has clarified and demonstrated that the steroid use is directly responsible for increased mortality. The study has also shown that the use of only DMARD therapy is associated with reduced risk than the combination therapy.<sup>4, 5</sup> The steroid use for managing RA was more prevalent in earlier days due to the

lack of in-depth understanding of the immunopathogenesis of RA, and pharmacokinetic and pharmacodynamics of these drugs.<sup>6</sup>

Steroid and several conventional DMARDs have been in clinical use for managing RA, with proven clinical efficacy, much before understanding their pharmacodynamics. There is limited literature evidence on the pharmacodynamic effects of DMARDs and immunosuppressive drugs. The in-depth knowledge on the immune response, both in normal and in pathologic processes like RA, has contributed to further understand the drug mechanisms. The DMARD and steroids confer both direct and indirect effects in altering the functioning of the immune and inflammatory pathways to achieve the effective control of autoimmune rheumatic diseases. The immunomodulators like steroids and methotrexate (MTX)

mediate the changes in immunological function through altering the gene expression or altering the function of primary or secondary cellular signaling pathways.<sup>7, 8</sup> There are studies reporting the *in vivo* and *in vitro* interactions of these drugs, and these interferences could be synergistic and/or antagonistic. For instance, there is a theoretical possibility for reduced efficiency of MTX-steroid combination therapy, as the MTX acts by inducing apoptosis through I $\kappa$ B kinase complex (IKK) pathway in activated lymphocytes.<sup>9</sup> Whereas, the steroids act by reducing the number of activated lymphocytes.<sup>10</sup> However, there is no adequate evidence on the reduced effectiveness of combination therapy in clinical studies. There are still more unanswered questions on the pharmacodynamic effects of these drugs including the drug interactions associated with sequential and concomitant use.

The major areas where the interactions of immunomodulators drugs can alter pharmacodynamics, and thereby the effectiveness, can be grouped into three domains. They are: 1. metabolic alterations, 2. immunological effects of the drugs and 3. gene induction or interference in gene expression of alternative or interdependent pathways. Within these three domains, interactions can be competitively exclusive or synergistically beneficial. The current review predominantly focuses on the molecular aspects of the pharmacodynamics of steroids and three commonly used DMARDs namely MTX, leflunomide and hydroxychloroquine. The metabolic interactions like enzyme induction are not included in the discussion.

### **The pharmacodynamics of steroids**

The pharmacokinetics and pharmacodynamics of both oral and intravenous prednisolone are dose dependent and have complex effects on the human immune response. The mechanism of the corticosteroid-induced immunoregulation is poorly understood. However, in recent years, there is better understanding of glucocorticoid (GC)-mediated immunosuppression with increased knowledge on the molecular, cellular, and pharmacological properties.

Administration of GC in a sustained fashion, throughout the course of the disease is considered to be highly beneficial in subjects with systemic inflammation. The timing of administration of GC is crucial and can explain some of the harmful effects associated with it.<sup>11</sup> Alternate-day corticosteroid therapy is widely used and has become a highly effective way of treating various conditions

like childhood nephrotic syndrome, chronic asthma, renal transplantation, and various inflammatory and autoimmune diseases.<sup>12-17</sup> This particular form of therapy minimizes the serious Cushingoid side effects and the incidence of infectious complications associated with daily prednisone therapy.<sup>18, 19</sup> However, the cutaneous delayed hypersensitivity remains intact.

Currently, the intracytoplasmic corticosteroid-specific receptors are considered as important pathways for steroid-induced changes.<sup>20</sup> Both endogenous and exogenous corticosteroids cause immunosuppression and increased incidence of infectious diseases.<sup>21</sup> Lymphopenia and eosinopenia are common findings after GC exposure.<sup>22</sup> Monocyte depletion in the blood stream is caused due to redistribution of cells into lymphoid tissues.<sup>23</sup>

A study by Fauci and Dale has addressed several concerns related to the circulating lymphocytes and monocytes of patients on alternate-day prednisone therapy. On the day of administration, the total circulating lymphocyte and monocyte counts were found to be normal in all the patients and in all the three dosage categories, immediately before the administration of prednisone at 8 am. After the administration of prednisone, transient but profound lymphocytopenia and monocytopenia were noted and were maximum after 4 hrs. These counts returned to normal 24 hrs and remained normal until the next dose of the alternate day. Thus, a transient cyclic decrease in circulating counts, persisting only for few hours, was noted every 2 days after the therapy. Same type of transient lymphocytopenia/monocytopenia was observed in normal volunteers after intravenous administration of a single dose of hydrocortisone.<sup>24</sup>

In an effort to study the effects of methylprednisolone on immune mechanisms in the absence of other immunologically mediated diseases or immunosuppressive agents, 17 normal adult male volunteers were administered with methylprednisolone 96 mg daily for 3-5 days in 6 divided doses and the results were compared with 12 untreated controls. Within several days after the methylprednisolone therapy, decrease in serum IgG concentration was noted in 12 subjects, decreased serum IgA in 6 and serum IgM in only 2 of the 14 treated volunteers. The rate of decrease of serum IgG declined after a week of cessation of corticosteroid treatment, suggesting that recovery began shortly after discontinuing the treatment. After 2-4 weeks of methyl prednisolone therapy, the treated volunteers had

22% mean decrease in IgG when compared to a decrease of only 1% in untreated controls. Significant decrease in IgA concentration was seen in 43% of the treated volunteers, whereas the decrease in IgM occurred only in 14% of the treated volunteers. After 3 days of methylprednisolone therapy, the lowest IgG levels were seen during the 2nd week after treatment; whereas after 5 days of therapy, the lowest IgG levels were noted during the 3rd and 4th week, and the magnitude of the drug effect was found to be dose-related. Increase in IgG concentration started earlier for the 3-day course compared to the 5-day course of treatment, indicating that IgG recovery is inversely related to the rate of fall in plasma drug levels. These findings reveal that the catabolism of IgG ceases within 2 days of discontinuation of methylprednisolone therapy and the drug-induced effect on plasma survival of IgG does not extend beyond 3 days after drug treatment.<sup>25</sup>

Haynes *et al.* have demonstrated that *in vivo* corticosteroids have a differential effect on the kinetics of subsets of cells in the human T-cell subsets.<sup>26</sup> Anthony and David have determined the effect of *in vivo* hydrocortisone on subpopulations of lymphoid cells in normal humans. Selective depletion of functional subpopulations of lymphocytes was noted. The phytohemagglutinin response was relatively not affected, while the response to concanavalin A was significantly reduced. Response to pokeweed mitogen remained unaffected by 100 mg of hydrocortisone but was diminished by 400 mg of hydrocortisone. *In vitro* responses to antigens like streptokinase-streptodornase and tetanus toxoid were reduced by *in vivo* hydrocortisone.<sup>24</sup>

A study conducted among 15 normal volunteers after a three-day course of prednisone 50 mg therapy, every 12 hours for six doses, noted the occurrence of transient

monocytopenia during the first few hours of therapy. Monocyte killing of *Staphylococcus aureus* was reduced in nine subjects and *Candida tropicalis* was reduced in four subjects ( $P < 0.01$ ). After 48 hours of dose of prednisone, bactericidal activity returned to normal. Normal or increased chemotactic response, phagocytic rate of cryptococci, hexose monophosphate-shunt response to phagocytosis and ultrastructural characteristics were seen with the same monocyte preparations. The impairment of bactericidal and fungicidal activity may be attributed to the development of infectious complications in patients receiving comparable doses of corticosteroids.<sup>27</sup> The effects of synthetic DMARDs and corticosteroids on different immune cells are provided in table 1.

### Pharmacodynamics of DMARDs

There is paucity of data pertaining to the pharmacodynamic effects of DMARDs like MTX, leflunomide and hydroxychloroquine. The studies on DMARDs are mainly focused on the impact of these drugs on various clinical and inflammatory parameters of the disease. The initial studies were primarily related to disease-specific features, joint counts, pain scale, and inflammatory parameters like ESR and CRP. These drugs are reported to alter several functional aspects of immune system response such as reducing the functioning of lymphocyte, neutrophil and antigen-presenting cells (Table 2).

**Methotrexate:** When MTX was introduced for the treatment of autoimmune rheumatic diseases, it was considered to be effective through its anti-folate and cytotoxic action. But the studies did not reveal significant association of cytotoxicity at lower weekly pulse dose. The mechanism involved could be multifaceted and include inhibition of purine and pyrimidine synthesis, suppression of transmethylation and reactions with accumulation of polyamines, and

**Table 1: The effects of synthetic DMARDs and corticosteroids on different immune cells**

Cells	MTX	LEF	HCQ	Cort
Neutrophil	+I	NA	+D	+D
Macrophage	+D	+D	+D	+D
Lymphocyte	NA	NA	+D	+D
B-cell	+D	NA	NA	+D
T-cell	+D	+D*	NA	+D
Synoviocyte	NA	+D	NA	+D
Antigen presenting cell	NA	+D	+D	NA

D direct effect, I indirect effect, NA data not available, + effect, \* inhibit TH2>TH1

**Table 2: Pharmacodynamic actions of steroids and DMARDs in RA**

Targets	Methotrexate	Leflunomide	Hydroxychloroquine	Glucocorticoids
NF-κB	Supresses the activation of NF-κB signalling pathway. <sup>9</sup>	Inhibits NF-κB activation. <sup>28</sup>	Supresses NF-κB activation. <sup>29</sup>	Inhibits NF-κB signalling pathway. <sup>30</sup>
T cell	<p>Inhibits lymphocyte proliferation.<sup>7</sup></p> <p>Leads to clonal deletion of activated T cells.<sup>7</sup></p> <p>Facilitates activation induced cell death.<sup>31</sup></p> <p>Inhibits the production of cytokines.<sup>32</sup></p> <p>Depletes the predominant CD4<sup>+</sup>CD28<sup>+</sup> and minor CD4<sup>+</sup>CD28<sup>-</sup> subpopulation in active RA patients.<sup>33</sup></p> <p>Supresses the expression of ICAM-1 and CLA in stimulated lymphocytes.<sup>34</sup></p> <p>Downregulates the incidence of CD25 phenotype.<sup>33</sup></p> <p>Prevents the activation of monocytes/macrophages.<sup>35</sup></p>	<p>Inhibits T cell activation and proliferation.<sup>28, 36-39</sup></p> <p>Inhibits IL-2 receptor expression and signalling.<sup>37, 40, 41</sup></p> <p>Inhibits T cell-dependent B cell formation of autoantibodies.<sup>42</sup></p> <p>Inhibits monocytic activation by stimulated T cells.<sup>43</sup></p>	<p>Inhibits T cell activation pathway.<sup>44</sup></p> <p>Induces apoptosis in peripheral blood T cells.<sup>45</sup></p> <p>Interacts with antigen processing in APCs.<sup>46</sup></p> <p>Inhibits TCR-mediated calcium mobilization and CD69 expression.<sup>44</sup></p> <p>Suppresses endosomal TLR activation.<sup>47</sup></p>	<p>Selectively depletes and supresses the differentiation of T lymphocyte.<sup>22, 48</sup></p> <p>Represses cytokine production of T lymphocytes (Th1 and Th2).<sup>48</sup></p> <p>Promotes the development of high IL-10-producing T cells.<sup>49</sup></p> <p>Dex induces TLR2 expression.<sup>50</sup></p>
B cell	-	Inhibits formation of IgA and IgG. <sup>42</sup>	-	<p>Promotes humoral immunity.<sup>49</sup></p> <p>Methylprednisolone decreases the plasma survival of IgG.<sup>25</sup></p>
Adenosine pathway	<p>Facilitates adenosine release.<sup>51</sup></p> <p>Modulates adenosine kinetics and dynamics.<sup>7</sup></p>	-	-	Upregulates the expression of A3adenosine receptor. <sup>52</sup>
Cytokine	<p>Inhibits the production of IL-4, IL-6, IL-13, TNF-α, IFNγ and granulocyte-macrophage colony-stimulating factor.<sup>32, 53-55</sup></p> <p>Inhibits the upregulation of IL-15, IL-6, IL-8, CD69, CD25, IFNγ and IL-17.<sup>56</sup></p>	<p>Inhibits IL1 and TNF-α.<sup>28</sup></p> <p>Suppresses IL-2 production.<sup>57</sup></p> <p>Augments TGFβ1.<sup>57</sup></p>	<p>Inhibits proinflammatory cytokines such as TNF-α, IFN-γ, IL-1-α and IL-6.<sup>58, 59</sup></p> <p>Reduces the levels of IL-6, IL-17 and IL-22.<sup>60</sup></p>	<p>Decreases IL-1β, IL-2 and IL-6 production.<sup>61-63</sup></p> <p>Inhibits IL-4 production.<sup>64</sup></p>

promotion of adenosine release with adenosine-mediated suppression of inflammation.<sup>65</sup> However studies based on *in vitro* and *in vivo* models have demonstrated several other pharmacodynamic effects of the drug (Table 2).

**Leflunomide:** Leflunomide is rapidly metabolized to its active form, A77 1726. The following two mechanisms

of action have been identified for A77 1726: inhibition of dihydroorotate dehydrogenase (DHODH) and inhibition of tyrosine kinases.<sup>38, 66</sup> DHODH inhibition, which is considered as the major mode of action, occurs at lower concentrations of A77 1726 than that of tyrosine kinases. Stimulated lymphocytes must increase ribonucleotide levels from 8 to 16-fold before proceeding to the S phase

**Table 3: Mechanism of action of steroids and DMARDs on NF- $\kappa$ B, T cell and adenosine pathway**

Targets	Drugs	Mechanism of action
NF- $\kappa$ B and activator protein-1 (AP-1)	Glucocorticoids	Binds to the p65 subunit of DNA-bound NF- $\kappa$ B molecule and inhibits the signalling pathway. <sup>30</sup>
		Decreases the activity of Akt and I $\kappa$ B kinase, essential for the phosphorylation and activation of NF- $\kappa$ B by associating with p85 $\alpha$ /P13K. <sup>70</sup>
		Inhibits NF- $\kappa$ B, by interacting with PKAc, crucial for the maximum transactivation capacity of NF- $\kappa$ B. <sup>71, 72</sup>
		Inhibits NF- $\kappa$ B signalling pathway by upregulating I $\kappa$ B $\alpha$ , cytoplasmic sequestration of p65, HDAC2 recruitment and interfering the phosphorylation status of RNA polymerase II. <sup>73</sup>
		Binds to c-Fos/c-Jun dimers of AP-1, thereby inhibiting their DNA binding and transactivation capacities. <sup>74</sup>
		Represses AP-1 activity by inhibiting the subsequent phosphorylation of c-Jun on S63/73. <sup>73</sup>
		Associates with JNK and loads inactive JNK to AP-1, while masking AP-1 from active JNK generated by MAPK activation. <sup>75</sup>
		The interaction of GR with MSK1 shifts the nuclear to cytoplasmic MSK1 ratio, thereby inhibiting the NF- $\kappa$ B activity. <sup>76</sup>
		GR dimers activate anti-inflammatory genes (Gilz, Annexin-1 and Mkp1) by binding to their respective GR response elements. <sup>77</sup>
		Interacts with coactivator molecules like CBP, pCAF, or SRCs, in the nucleus and activates anti-inflammatory genes (including Spli, Mkp1/Dusp1, I $\kappa$ B- $\alpha$ , and Gilz). <sup>78, 79</sup>
		GR dimerization inhibits JNK2, a crucial mediator of TNF-induced inflammation, through Mkp1. <sup>80</sup>
		Peroxisome proliferator-activated receptor $\gamma$ (PPAR $\gamma$ ) interacts with GR and they conjointly act as immunosuppressors. <sup>75</sup>
		During stressful events, GR induces c-Fos and early growth response protein (Egr-1) by increasing the phosphorylation of ERK. <sup>81</sup>
		The simultaneous activation of PPAR $\alpha$ and GR enhances transrepression of NF- $\kappa$ B-driven gene expression and represses cytokine production. <sup>82</sup>
		Downregulates NF- $\kappa$ B activity and subsequent generation of cytokines (IL-1 $\beta$ , IL-2, IL-6), by modulating the expression of zinc transporter. <sup>61-63</sup>
		Competes with NF- $\kappa$ B for coactivators like CBP and SRC-1, required for their induction of downstream genes/transactivation. <sup>83</sup>
		The binding of GR with corepressors like GRIP1, results in the transrepression of the target proinflammatory cytokine genes of AP-1 and NF- $\kappa$ B. <sup>84</sup>
	Methotrexate	Prevents the activation of NF- $\kappa$ B signalling pathway by inhibiting the degradation and suppressing the phosphorylation of I $\kappa$ B $\alpha$ . <sup>9</sup>  Supresses the expression of receptor activator of NF- $\kappa$ B ligand, RANKL and RANKL mRNA, thereby indirectly inhibiting osteoclast formation. <sup>85</sup>
	Leflunomide	A77 1726 (active metabolite of leflunomide), inhibits NF- $\kappa$ B activation by targeting the degradation of inhibitory protein I $\kappa$ B $\alpha$ . <sup>28</sup>
	Hydroxychloroquine	Hydroxychloroquine HCQ suppresses IL-1 $\beta$ -induced activation of NF- $\kappa$ B. <sup>29</sup>

Targets	Drugs	Mechanism of action
T cell	Glucocorticoids	<p>The binding of GCs to the GR limits the availability of unliganded GR, essential for the efficient TcR signalling and Tc R-dependent LCK/FYN activation.<sup>86</sup></p> <p>GR interacts with NF-AT (nuclear factor of activated T cells), and inhibits the 1L-4 production of T cells.<sup>64</sup></p> <p>GCs interferes with T-bet and GATA-3 and downregulates the cytokine production of Th1 and Th2 respectively.<sup>48</sup></p> <p>GR interferes with the TLR3/4 signalling cascade by competing with IRF3 for binding to GRIP1.<sup>87</sup></p> <p>Methylprednisolone facilitates the catabolism of IgG, thereby decreasing its plasma survival.<sup>25</sup></p> <p>Dex induces TLR2 expression by recruiting GR and STAT5 at the TLR2 promoter <i>in vivo</i>.<sup>50</sup></p>
	Methotrexate	<p>Prevents the <i>de novo</i> pyrimidine and purine synthesis, thereby inhibiting the cellular proliferation of T cells.<sup>7</sup></p> <p>MTX and/or MTX-polyglutamates inhibit enzymes such as dihydrofolate reductase, thymidylate synthase and 5-aminoimidazole-4-carboxamide ribonucleotide transformylase.<sup>88, 89</sup></p> <p>Facilitates clonal deletion of activated T cells via ROS-dependent and mitochondria-mediated pathways.<sup>7, 90</sup></p> <p>Increases the homocysteine levels and subsequently reduces the methylation of Ras protein, thereby executing anti-proliferative effect.<sup>91</sup></p> <p>Increases CD95 sensitivity and facilitates activation-induced cell death.<sup>31</sup></p> <p>Inhibits the upregulation of IL-15, IL-6, IL-8, CD69, CD25, IFN<math>\gamma</math> and IL-17, and disrupts the interaction between synovial fibroblasts and T lymphocytes.<sup>56</sup></p> <p>Interacts with several agonists and suppress IL-6 production in osteoblastic cell lines.<sup>92</sup></p>
	Leflunomide	<p>Prevents T cell division primarily by inhibiting <i>de novo</i> pyrimidine ribonucleotide synthesis in the late G1 phase and at higher doses by inhibiting tyrosine kinases.<sup>93, 39</sup></p> <p>Non-competitively inhibits dihydroorotate dehydrogenase, thereby preventing <i>de novo</i> pyrimidine synthesis.<sup>42</sup></p> <p>Causes cell cycle arrest at the late G1 phase by decreasing rUMP levels and upregulating p53 and p21.<sup>94, 95</sup></p> <p>Inhibits the activities of the Src-related tyrosine kinases p56/<i>ck</i> (reduces IL-2 production) and p59/<i>fyn</i>, in Jurkat T cells.<sup>40</sup></p> <p>Effectively inhibits the levels of tyrosine phosphorylated proteins, in mouse leukemia cell line (LSTRA) cells, which overexpress p56/<i>ck</i>.<sup>96</sup></p> <p>Inhibits the phosphorylation of Jak1 and Jak3 tyrosine kinases, which are necessary for IL2 receptor signalling.<sup>41</sup></p>
	Hydroxychloroquine	<p>Inhibits T cell stimulation by inhibiting the digestion of antigenic proteins and the assembly of peptides with the <math>\alpha</math> and <math>\beta</math> chains of MHC class II proteins in APCs.<sup>46, 97</sup></p> <p>Blocks the proliferative responses of T-cell mitogens and alloantigens.<sup>44</sup></p> <p>Decreases the production of TNF-<math>\alpha</math> in human macrophages, and not of monocytes and T cells.<sup>98, 99</sup></p>



		<p>Induces apoptosis in peripheral blood T lymphocyte through caspase cascades.<sup>45</sup></p> <p>Decreases the levels of IL-6, IL-17 and IL 22, possibly by reducing Th17 cells, through a decrease in antigen presentation.<sup>60</sup></p> <p>Inhibits TNF<math>\alpha</math>, IL-1<math>\beta</math> and aPL-mediated induction of endosomal NOX, in human monocytes and MonoMac1 cells by inhibiting the translocation of gp91phox, catalytic subunit of NOX2.<sup>100</sup></p>
Adenosine pathway	Glucocorticoids	GCs up-regulates the expression of A3 adenosine receptor, resulting in the activation of extracellular signal regulated kinase (ERK)1/2 phosphorylation, thereby promoting survival of anti-inflammatory macrophages. <sup>52</sup>
	Methotrexate	<p>Increases adenosine levels by decreasing the activity of purine enzymes adenosine deaminase, purine-nucleoside phosphorylase and hypoxanthine-guanine-phosphoribosyl transferase.<sup>101</sup></p> <p>Stimulate A2a-receptor through adenosine release, resulting in anti-inflammatory effects.<sup>102</sup></p> <p>Significantly suppresses NURR1 expression in patients with psoriatic arthritis, mediated through the adenosine receptor A2.<sup>7, 103</sup></p> <p>Reduces the adherence of T lymphocytes to synovial fibroblasts, mediated by adenosine release.<sup>56</sup></p>
	Leflunomide	---
	Hydroxychloroquine	---

from G1. Increased levels of ribonucleotides can only be met by *de novo* ribonucleotide synthesis. At low levels of ribonucleotides, p53, the activation of a 'sensor' molecule prevents progression through the cell cycle. Therefore, an inhibitor of *de novo* uridine monophosphate synthesis would predictably arrest stimulated cells at the G1 phase.<sup>67</sup> Mice model studies suggest the inhibition of tyrosine kinase as the more active mechanism than dihydroorotate dehydrogenase (DHODH) inhibition.<sup>68</sup> Inhibition of pyrimidine synthesis promotes TH2 cell activation and inhibition of TH1 cell activity.<sup>40</sup> Its effects on different cells are summarized in table 2.

Hydroxychloroquine is considered as a potential immunomodulator interfering the antigen presentation and expression of several receptors (Table 2). The drug is effective after 6 to 8 weeks of loading dose, suggesting the cumulative effect of the drug.<sup>69</sup> Though the exact mechanism of the drug is uncertain, clinical studies have demonstrated the advantage of combining the drug with other DMARDs. The molecular mechanism of the drug is given in table 3.

### Drug interactions in pharmacodynamics

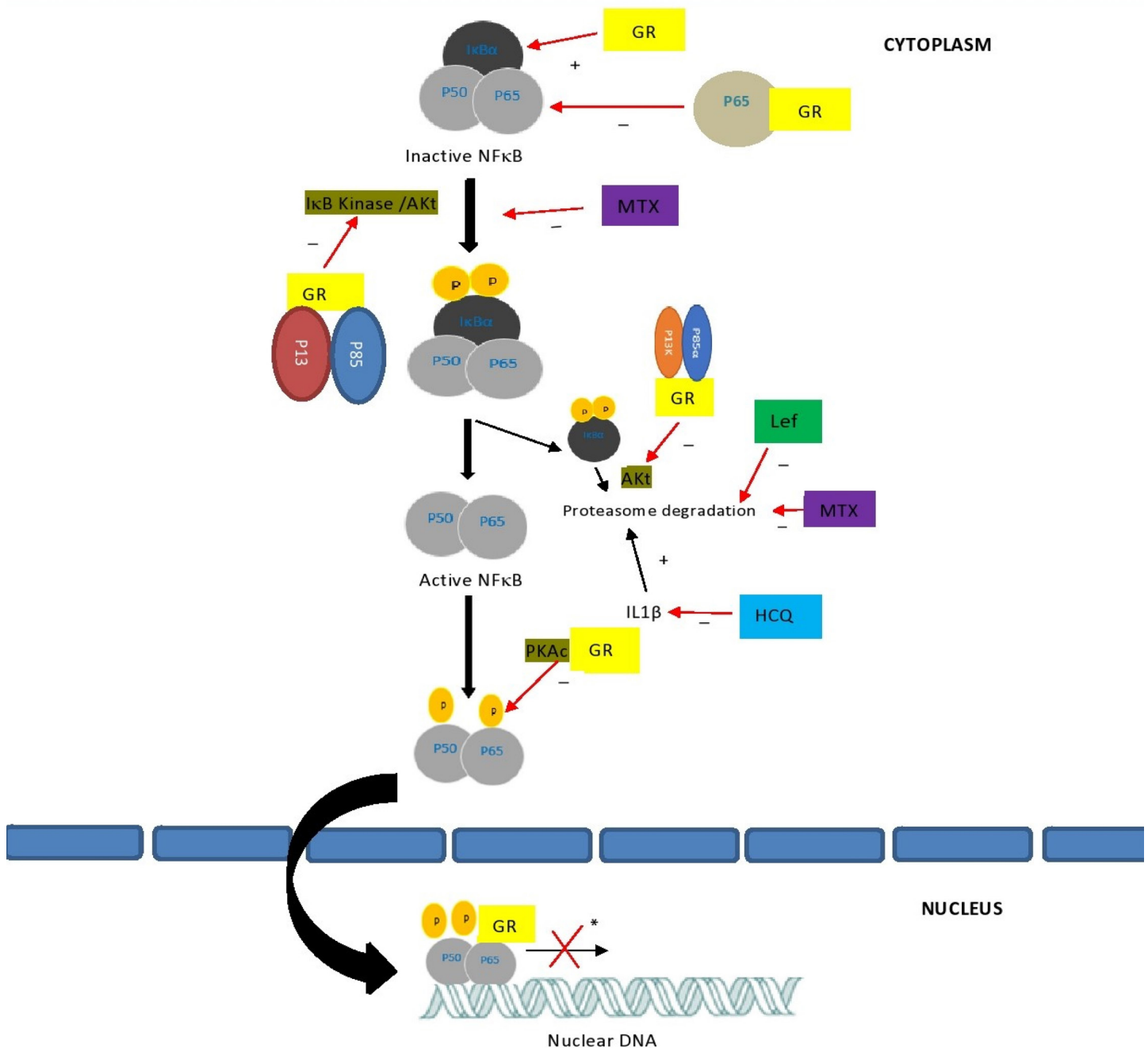
The drugs like glucocorticoids, MTX, leflunomide and

hydroxychloroquine alter the immune system function by interfering both metabolic and secondary signal pathways, thereby altering the gene expression. The mechanism of interference in cell signaling as well as in metabolic processes of individual drug is shown in table 3. Here we have attempted to show the three major pathways namely NF- $\kappa$ B (Fig. 1), T cell (Fig. 2A & 2B) and adenosine pathways (Fig. 3). The figures also depict the sites of convergence of actions of different drugs. Both MTX and leflunomide act at different levels of metabolic pathways of purine and pyrimidine synthesis (Fig. 2A).<sup>104</sup> The interactions seem to be clinically synergistic.<sup>105</sup> The interactions of DMARDs and steroid suggest initial advantage in improving the disease control. However, with respect to improved survival, there may not be significant advantage in long term.

### Combination pharmacodynamics

With respect to the use of combination drugs, one of the major challenges confronted by clinicians is whether to introduce drugs simultaneously or in step-wise manner. In combination treatment, the doses can be altered to receive the optimal dose of each component, to retain the efficacy and to reduce the incidence of adverse events associated with the individual use. For predicting the quantitative effects of synergisms of the drugs, methods

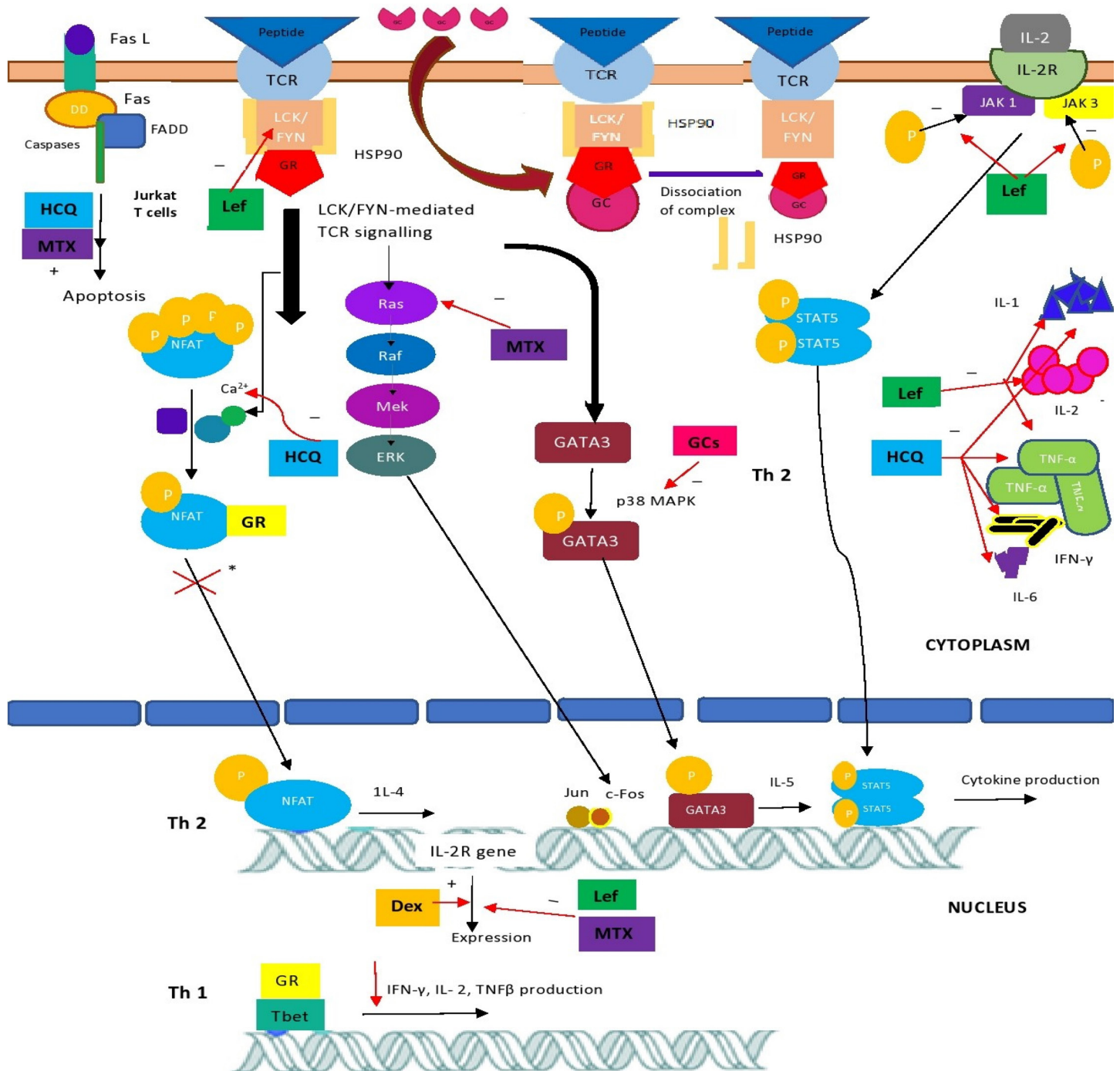
**Fig. 1: Major targets of action of steroids and DMARDs on NFκB signaling pathway**



GR binds to the p65 subunit of DNA-bound NFκB molecule and inhibits the signalling; GR associates with P13K/p85α and decreases the activity of Akt and IκB kinase, which is essential for the phosphorylation and activation of NFκB; GR interacts with PKAc and inhibits NFκB; GR inhibits NF-κB pathway by upregulating IκBα and by facilitating cytoplasmic sequestration of p65; GR and Lef inhibit the proteasomal degradation of phosphorylated IκBα; MTX inhibits the degradation and suppresses the phosphorylation of IκBα; HCQ suppresses IL-1β-induced activation of NF-κB.



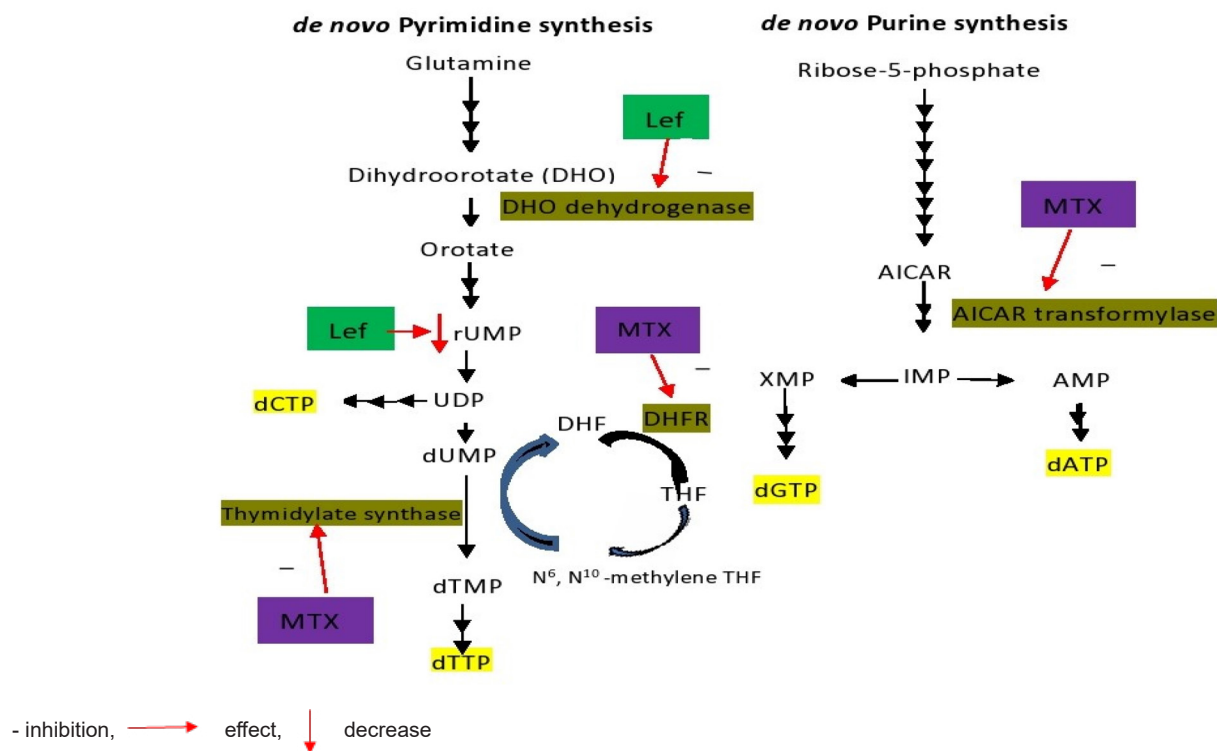
**Fig. 2A: Targets of action of steroids and DMARDs on T cells**



+ induction, - / \* inhibition, → effect

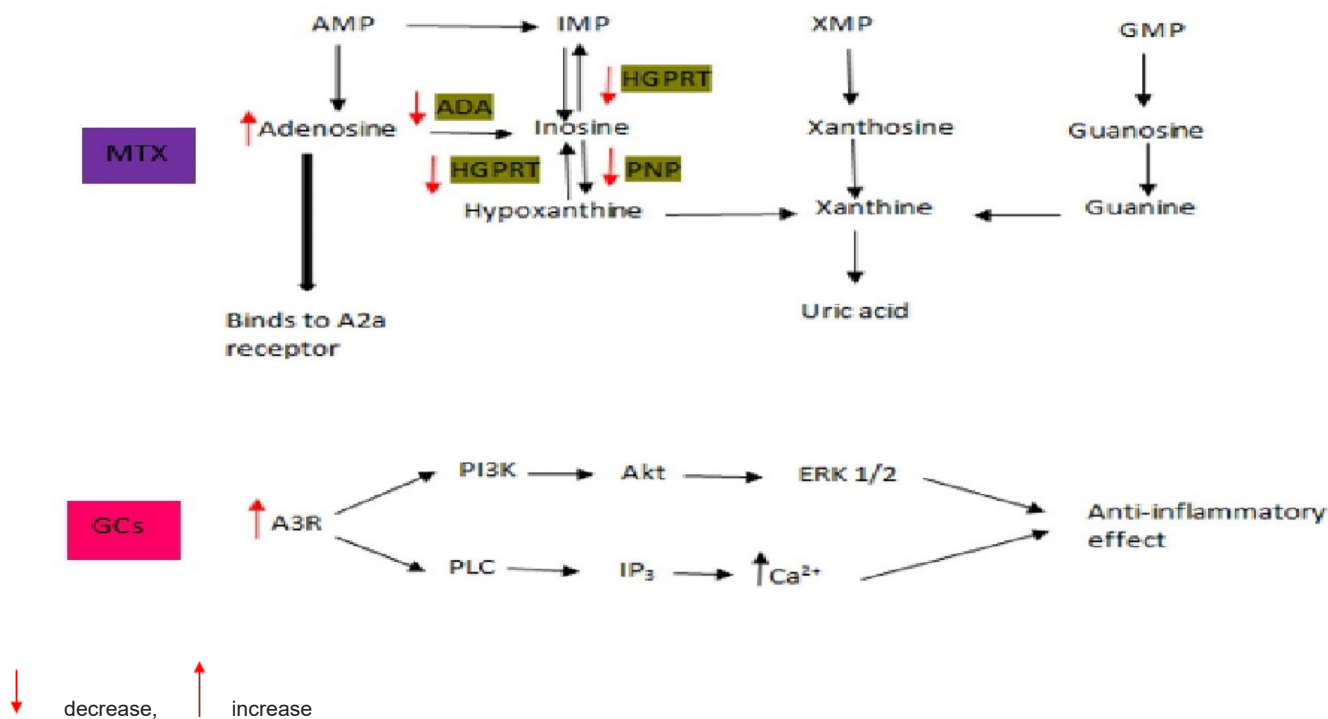
GCs inhibits TCR signalling and TCR-dependent LCK/FYN activation by binding to the free GR required for efficient signalling; GCs downregulates the cytokine production in Th1 and Th2 cells by interfering with Tbet and GATA-3 respectively; GR inhibits IL-4 production in T cells by interacting with NFAT; MTX increases the homocysteine level and subsequently reduces the methylation of Ras proteins; MTX facilitates activation-induced cell death; MTX and Lef inhibit the expression of IL-2R gene; Lef prevents IL2 receptor signalling by inhibiting the phosphorylation of JAK 1 and JAK 3 tyrosine kinases; Lef inhibits the activity of p56lck in Jurkat T cells and also inhibits cytokines such as IL1 and TNF- $\alpha$ , and suppresses IL2 production; HCQ inhibits TCR-mediated calcium mobilization and induces apoptosis in peripheral blood T cells through caspase cascade; HCQ also inhibits cytokines like TNF- $\alpha$ , IFN- $\gamma$ , IL6 and IL-1- $\alpha$ .

**Fig. 2B: Targets of action of DMARDs on *de novo* purine and pyrimidine synthesis**



MTX inhibits enzymes like DHFR, thymidylate synthase and AICAR transformylase involved in *de novo* purine and pyrimidine biosynthesis; Lef prevents *de novo* pyrimidine synthesis by non-competitively inhibiting DHO dehydrogenase and decreasing rUMP level, thereby causing cell cycle arrest at late

**Fig. 3: Targets of action of steroids and DMARDs on adenosine pathway**



MTX increases adenosine release by decreasing the activity of ADA, PNP and HGPRT; GCs upregulates the expression of A3 adenosine receptor and promotes the survival of anti-inflammatory macrophages.

such as combination index are used.<sup>106</sup> The simple principle of the combination treatment is that if the drugs are competing for same pathways, the efficacy is not the sum of their individual effect and the combined use may not confer any advantage. Evaluation of the mechanisms and pharmacodynamic effects of the steroid and other DMARDs reveals several instances of overlap in gross pharmacodynamic action on lymphocyte, inhibition of NF- $\kappa$ B and other intervention pathways. But the sites of action in the cell signal pathways of these drugs are different with a few convergent locations (Fig. 1). Analysis of intervention in NF- $\kappa$ B pathway of steroid, leflunomide and MTX shows that the step interfered by leflunomide is prior to that of MTX, whereas steroids act at multiple steps. Blocking of the downstream pathways by the drugs is expected to have different impact when compared to upstream pathway blocking. For instance, if the blocking at downstream pathway increases the number of functional receptors through counter-regulatory mechanism, higher concentrations of drugs levels are needed to prevent the repercussions. Whereas the downstream blocking reduces the number of receptors, it may enhance the response even at lower concentrations. However, the available evidence on the intervention of upstream/downstream pathways from the *in vitro* and *in vivo* studies is very limited, further research in this area is warranted to improve the use of combination and also for personalizing the DMARD and steroid prescriptions. Studies involving *in silico* and experimental models are necessary to clearly understand these drugs interactions, which may facilitate the improvement of treatment strategies and customization of treatment approaches.

### Abbreviations

MTX, methotrexate; LEF/Lef, leflunomide; HCQ, hydroxychloroquine; Cort, corticosteroids; GR, glucocorticoid receptor; GC, glucocorticoids; Dex, Dexamethasone; PKAc, catalytic subunit of protein kinase A; Th 1, type 1 T helper cells; Th 2, type 2 T helper cells; TCR, T cells receptor; DHFR, dihydrofolate reductase; AICAR transformylase, 5-aminoimidazole-4-carboxamide ribonucleotide transformylase; dTTP, deoxythymidine triphosphate; dCTP, deoxycytidine triphosphate; dGTP, deoxyguanosine triphosphate; dATP, deoxyadenosine triphosphate; rUMP, ribonucleotide uridine monophosphate; ADA, adenosine deaminase; HGPRT, hypoxanthine-guanine phosphoribosyltransferase; PNP, purine nucleoside phosphorylase; A3R, adenosine 3 receptor; A2a receptor, adenosine 2a receptor.

### Competing interests

The author declares that he has no competing interests.

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