Molecular mechanisms of glucocorticoid action and selective glucocorticoid receptor agonists
Cindy Stahn, Mark Löwenberg, Daniel W. Hommes, Frank Buttgereit

To cite this version:
Cindy Stahn, Mark Löwenberg, Daniel W. Hommes, Frank Buttgereit. Molecular mechanisms of glucocorticoid action and selective glucocorticoid receptor agonists. Molecular and Cellular Endocrinology, Elsevier, 2007, 275 (1-2), pp.71. 10.1016/j.mce.2007.05.019. hal-00531935

HAL Id: hal-00531935
https://hal.archives-ouvertes.fr/hal-00531935
Submitted on 4 Nov 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
Molecular mechanisms of glucocorticoid action and selective glucocorticoid receptor agonists

Cindy Stahn¹,⁴, Mark Löwenberg², Daniel W. Hommes³, Frank Buttgereit¹

¹ Department of Rheumatology and Clinical Immunology
Charité University Hospital
Schumannstrasse 20/21
10117 Berlin
Germany

² Department of Gastroenterology and Hepatology
Academic Medical Center
Meibergdreef 9
NL-1105 AZ Amsterdam
The Netherlands

³ Department of Gastroenterology and Hepatology
Leiden University Medical Center
Albinusdreef 2
NL-2300 RC Leiden
The Netherlands

⁴ To whom correspondence should be addressed
Abstract

Glucocorticoids (GC) are the most common used anti-inflammatory and immunosuppressive drugs in the treatment of rheumatic and other inflammatory diseases. Their therapeutic effects are considered to be mediated by four different mechanisms of action: the classical genomic mechanism of action caused by the cytosolic glucocorticoid receptor (cGCR); secondary non-genomic effects which are also initiated by the cGCR; membrane-bound glucocorticoid receptor (mGCR)-mediated non-genomic effects; and non-specific, non-genomic effects caused by interactions with cellular membranes. The classical, genomic mechanism of GC-action can be divided into two processes: “transrepression”, which is responsible for a large number of desirable anti-inflammatory and immunomodulating effects, and “transactivation” which is associated with frequently occurring side effects as well as with some immunosuppressive activities [1]. Great efforts have been made to diminish glucocorticoid-induced adverse effects, but the improvement of conventional glucocorticoids has almost reached its limits. As a consequence, new variations of the conventional “good old drugs” are being tested and nitro-steroids and long circulating liposomal glucocorticoids indeed show promising results. Nevertheless, crux of the matter should be the design of qualitatively new drugs such as selective glucocorticoid receptor agonists (SEGRA’s). These innovative steroidal or non-steroidal molecules induce transrepression, while transactivation processes are less affected. First reports on two different GCR ligands, A276575 and ZK216348, show promising results. Here, we review the above mentioned mechanisms of glucocorticoid action and give particular attention to the development of optimized glucocorticoids and SEGRA’s.
Introduction

Glucocorticoids are successfully used in the treatment of a wide range of rheumatic and other inflammatory diseases. Clinically relevant in this regard are immunosuppressive, anti-inflammatory and anti-allergic effects that glucocorticoids exert on primary and secondary immune cells, tissues and organs. The most important effects of glucocorticoids on different cell types are listed in table 1 [2].

The inactive form of the glucocorticoid cortisol, so-called cortisone, has been isolated in the years 1936-1940 by different groups, and cortisol was first synthesised by Reichstein in 1937/1938. About ten years later, glucocorticoids were introduced into clinical medicine and the researchers primarily involved were awarded the Nobel price for their fundamental work. Since then, glucocorticoids were more and more used to treat numerous diseases, but at the same time the potential of these drugs to induce unwanted effects became obvious. This downside was the reason why new glucocorticoid agents were synthesised in the 1950-60’s, e.g. prednisolone and methylprednisolone having stronger anti-inflammatory and immunosuppressive potencies, and lesser mineralcorticoid activities. Other examples for synthetic drugs are the fluorinated glucocorticoids dexamethasone and betamethasone.

Another advancement was the delivery of these drugs directly to the side of inflammation, e.g. by inhalation in case of asthma, by topical application for eczema or in the case of rheumatic diseases by intra-articular injection. Glucocorticoids are relatively inexpensive drugs, but due to the enormous volume prescribed, the total market size is believed to be about 10 billion US dollars per year [3].

In spite of every effort to improve treatment with glucocorticoids, these drugs still carry significant risks [4]. Treatment with higher glucocorticoid dosages over longer periods of time causes adverse reactions such as unwanted effects on metabolism (diabetes), bone tissue (osteoporosis), muscles (myopathy), eyes and skin [3,5]. Furthermore, glucocorticoids may cause increased susceptibility to infections. Another major problem in the treatment of
inflammatory diseases represents glucocorticoid resistance [6,7]. In order to optimise the
benefit-risk-ratio it is important to understand better the underlying mechanisms of
glucocorticoid action. Different studies have shown that glucocorticoid activities can be
divided into genomic effects, mediated by the cytosolic glucocorticoid receptor alpha (cGCR),
and different non-genomic effects [2,6-12]. These non-genomic glucocorticoid activities can
be subclassified further into three modes of action: cGCR-mediated non-genomic effects;
non-specific non-genomic effects (for example physicochemical interactions with the plasma
membrane at high glucocorticoid concentrations); and effects that are considered to be
mediated by membrane-bound glucocorticoid receptors [9,13].
The classical genomic mechanism of glucocorticoid action is cGCR-mediated

The unligated cytosolic glucocorticoid receptor, a member of the steroid-hormone-receptor family, is a 94-kd protein which exists in the cytoplasm as a multiprotein complex containing several heat-shock proteins (Hsp), such as Hsp90, Hsp70, Hsp56 and Hsp40. There is also an interaction with immunophilins, (co)chaperones (such as p23 and Src [7,8,11]), and several kinases of the mitogen-activated protein kinase (MAPK) signalling system. The glucocorticoid receptor consists of three different domains with various functions: an N-terminal domain containing transactivation functions, a DNA binding domain that implies a zinc-finger motif (e.g. a motif that is common to DNA interaction proteins) and a ligand-binding domain consisting of 12 α-helices which is involved in the formation of the hydrophobic ligand-binding pocket [7]. Glucocorticoids can easily pass through the plasma membrane due to their lipophilic structure. The inactive glucocorticoid receptor is able to bind different glucocorticoids with high affinity. Formation of the activated GC/cGCR complex results in dissociation of the above mentioned (co)chaperones from the glucocorticoid receptor [8]. This receptor complex is then translocated into the nucleus within 20 minutes, where it binds as a homodimer to specific DNA binding-sites (i.e. glucocorticoid responsive elements, GREs) [8]. Subsequent binding of the ligand-activated glucocorticoid receptor to positive GREs results in induced synthesis of anti-inflammatory proteins (e.g. lipocortin 1, IκB), but also regulator proteins (Figure 1, mechanism I) that are important for metabolism (for example enzymes involved in gluconeogenesis), thereby exerting many different effects on the cellular, tissue, organ and organism level. This process, which is mediated via positive GREs, is termed “transactivation”, and is thought to be responsible for numerous side effects of glucocorticoids. Transcription of genes can be inhibited by GCs via direct interaction between the GCR and negative GRE’s, such as the pro-opiomelanocortin, α-fetoprotein and
prolactin gene [14]. In line with this notion, it has been shown that glucocorticoids suppress transcription of inflammatory genes, including interleukin (IL)-1 and IL-2, via negative GREs [15,16] (Figure 1, mechanism II). However, the exact role of nGREs in mediating GC-induced effects in cellular systems remains largely unclear. Alternatively, transcription factors can be displaced from their positive GRE, through direct protein-protein interaction between transcription factors and GCR.

The term “transrepression” refers to molecular mechanisms in which monomers of the glucocorticoid/glucocorticoid receptor complex directly or indirectly interact with transcription factors (Figure 1, mechanism III), such as activator protein 1 (AP1), nuclear factor-κB (NF-κB) or interferon regulatory factor-3 (IRF-3) [18], transcription factors which are involved in regulating the expression of pro-inflammatory genes. Negative regulation by glucocorticoid/glucocorticoid receptor complexes results in reduced transcriptional activities of GCR target genes [19-23], leading to anti-inflammatory and immune-suppressive effects [24]. In this way glucocorticoids inhibit nuclear translocation and the function of several pro-inflammatory transcription factors, thereby suppressing synthesis of inflammatory mediators, including cytokines (e.g. IL1, IL2, TNF-α, IFN-γ) and prostaglandins. The following underlying mechanisms have been suggested: 1. Prevention of transcription factor binding to specific DNA sequences through direct protein-protein interaction; 2. Induced synthesis of IκB, an inhibitor of NF-κB, caused by the interaction between GRE and the GC/cGCR complex; 3. Competition for nuclear coactivators between the GC/cGCR complex and transcription factors (Figure 1, mechanism IV).

More than 30 minutes pass before significant changes are recognized in regulator protein concentration. This time is needed for activation of the cGCR, nuclear transportation of the receptor complex, binding to GREs in the promoter regions of target genes and the initiation of transcriptional and translational processes leading to newly synthesised proteins. It usually takes hours or days before changes on cellular, tissue or organism level become evident.
However, some of the immunosuppressive, anti-inflammatory and anti-allergic glucocorticoid effects occur too fast to be explained by the classical, genomic mechanism of glucocorticoid action [9,15,25-27]. For example, rapid clinical effects have been observed when glucocorticoids were administered intravenously or intra-articularly at high dose. Over the last years, great efforts have been made in order to understand the mechanisms responsible for rapid glucocorticoid-induced effects.
Rapid effects of glucocorticoids are mediated by three different mechanisms

Three different non-genomic mechanisms have been proposed to explain rapid anti-inflammatory and immunosuppressive GC effects [9,15,25-27]:

- non-specific interactions of glucocorticoids with cellular membranes,
- non-genomic effects which are mediated by the cytosolic GR (cGCR),
- specific interactions with a membrane-bound GCR (mGCR),

We will now discuss these mechanisms that underlie nongenomic GC activities.

Glucocorticoids interact with cellular membranes

Glucocorticoids have rapid effects on biological membranes, especially plasma and mitochondrial membranes. It has been shown that GCs at high concentration intercalate into membranes thereby changing their physicochemical properties as well as activities of membrane-associated proteins [9,10]. This results in reduced calcium and sodium cycling across plasma membranes of immune cells, which is thought to contribute to rapid immunosuppression and a subsequent reduction of the inflammatory process [9]. These effects are caused by direct membrane effects, rather than by reduced ATP production [9,10,28]. Furthermore, mitochondrial proton leak has been found to be increased by glucocorticoids resulting in impaired ATP production. These rapid GC effects on mitochondrial membranes may contribute to clinically relevant outcomes, since ATP is vital for housekeeping activities of immune cells as well as their specific effector functions, such as cytokine synthesis, migration, phagocytosis, antigen processing and antigen presentation.

Non-genomic glucocorticoid effects caused by cGCR-binding
Nongenomic glucocorticoid effects might also be mediated by the cGCR following GC/cGCR binding. As mentioned before, the unligated GC receptor is located in the cytoplasm as a multi-protein complex consisting of heat-shock proteins and several kinases (such as MAPKs). Due to GC receptor ligand binding, the cGCR becomes dissociated from this protein complex resulting in GCR nuclear translocation. The release of signalling molecules from the GCR-multi-protein complex due to GCR ligand binding, such as Src, is considered to be responsible for rapid GC effects [26]. Another observation concerns arachidonic acid (AA), an essential mediator for cell growth and several metabolic/inflammatory reactions. Release of AA from cell membrane-associated phospholipids is controlled by different mediators (such as growth factors, adaptor proteins, MAPK and lipocortin 1) and can be inhibited by glucocorticoids by a cGCR-dependent (i.e. RU486-sensitive) but transcription-independent (i.e. actinomycin D-insensitive) mechanism [26]. Hence, data indicate that cGCR is not only important as a nuclear regulator of gene transcription, but is also involved in rapid nongenomic GC-induced effects.

**Specific non-genomic glucocorticoid effects mediated by a membrane-bound glucocorticoid receptor**

A third hypothesis which might explain non-genomic GC effects on immune cells involves specific effects of GCs mediated by a membrane-bound glucocorticoid receptor (mGCR). The existence of this receptor had been shown for the first time in amphibian neuronal membranes and in lymphoma cells [29]. mGCR on human peripheral blood mononuclear cells were identified using high-sensitive immunofluorescent staining [10,28,30]. The advantage of this method is to increase fluorescence intensity up to 1000-fold as compared with conventional imaging methods by using antibody conjugated magneto-fluorescent liposomes. It is therefore possible to detect 50-100 target molecules per cell. The monoclonal antibody used for the detection recognises not only cGCR but also mGCR. Overexpression of cGCR did not show
an increased mGCR expression on the cell surface. Hence, it is assumed that mGCR is not just an unchanged cGCR that has been transported to the cell surface. It is rather suggested that the mGCR is a variant of cGCR produced by differential splicing or promoter switching or by post-translational editing [30]. The origin of this receptor still remains unexplained, but further experiments are currently being made to prove the so called “one-gene-hypothesis”.

We have reported that stimulation with lipopolysaccharide (LPS) increases the percentage of mGCR positive monocytes, which could be prevented by inhibiting the secretory pathway using brefeldin A. It is, therefore, suggested that immunostimulation is responsible for up-regulation and trans-cellular transport of mGCR [30]. Clinical data provided further evidence for this view. As one example, it has been shown in patients with rheumatoid arthritis (RA) that an increased number of mGCR positive monocytes and B-lymphocytes correlates with different parameters of disease activity (i.e. higher disease activity scores correlate with increased mGCR-positive cell numbers) [2,30]. Expression of mGCR is also up-regulated in monocytes and in B-lymphocytes from patients with ankylosing spondylitis, but this up-regulation does not correlate with disease activity [31]. Finally, patients with systemic lupus erythematosus show high/increased numbers of mGCR positive monocytes, sometimes even higher than found in patients with active RA [13]. These results demonstrate that mGCR may play a role in the pathogenesis of chronic inflammatory diseases. Since glucocorticoid-induced mGCR-mediated apoptosis has been reported [29], the up-regulation of mGCR due to immunostimulation or in case of disease activity could be considered as a protective mechanism. Perhaps, mGCR may serve as drug targets, but the origin, the detailed mechanism of action and function of mGCR should be clarified first. One approach to clarify the origin of the mGCR might be the use of e.g. RNAi technology in order to investigate the effect of cGCR knock-down on mGCR expression. To shed light on the functional role of mGCR, the effect of selective binding of GC to mGCR (employing BSA-bound GC compounds) on cellular signalling events should be studied.
Recent work provided molecular insight into nongenomic immunosuppressive effects of GCs in T cells, mediated by a membrane-linked GC receptor. p56lck (Lck) and p59fyn (Fyn) kinases were identified as cellular targets for nongenomic GC activities [32]. Lck and Fyn are members of the Src family of tyrosine kinases, expressed in T cells, and they are involved in T cell receptor (TCR)-mediated signal transduction [33-35]. Their association with the TCR complex (Lck binds to CD4 or CD8 co-receptors, whereas Fyn associates with CD3) and the cellular organisation of Lck and Fyn is essential for efficient TCR signalling [36,37]. The quality of TCR signalling is predominantly influenced by the TCR-CD4 association, Lck being the critical mediator [38], leading to activation of signal transduction cascades downstream of the TCR. We have recently investigated rapid GC effects on the CD4⁺T-cell signal transduction kinome using a peptide array containing 1176 different kinase consensus substrates [32]. These experiments revealed 116 kinase substrates with either increased or decreased phosphorylation due to short-term GC treatment. Among the most prominent effects observed was decreased phosphorylation of Lck/Fyn consensus substrates. It was demonstrated that GC treatment rapidly inhibits Lck and Fyn activities \textit{in vitro} and \textit{in vivo}, mediated via a GCR-dependent pathway [32]. Based on further studies, a molecular mechanism for GC-induced inhibition of TCR signalling has been proposed [39]. It was shown that the GCR is a part of a TCR-linked multi-protein complex containing HSP90, Lck and Fyn. Experiments employing siRNA constructs revealed that both the GC receptor as well as HSP90 are essential components of the TCR signalling complex. Altogether, these studies showed that short-term treatment with the GC analogue dexamethasone disrupts TCR-linked GCR multi-protein complexes followed by a cellular redistribution of Lck and Fyn kinases and in impaired TCR signalling [39].

\textbf{Optimized glucocorticoids and selective glucocorticoid receptor agonists}
The different molecular mechanisms of glucocorticoid actions afford a certain number of starting points for the development of optimized and/or new glucocorticoids and glucocorticoid receptor ligands.

**Long circulating liposomal glucocorticoids**

The first interesting development concerns the optimized usage of conventional glucocorticoids: the targeted delivery of these drugs by means of a carrier system in the form of liposomes. Liposomes are small vesicles about 100 nm in size, consisting of a lipid bilayer and a hydrophilic core, and glucocorticoids can be encapsulated into liposomes. To prevent a breakdown by the mononuclear phagocyte system in liver and spleen, hydrophilic polymers (e.g. polyethylene glycol) are associated to the surface of liposomes. Liposomal glucocorticoids accumulate directly at the site of inflammation, accordingly very high local concentrations are reached and, thus, they are therapeutically superior to conventional intravenous high-dose glucocorticoid therapy [40]. A recent study showed repeated daily injection of the same concentration (10 mg/kg) of non-capsulated drug to be less effective than a single injection of liposomal prednisolone which resolved joint inflammation in mice for a week [41]. In addition, fewer adverse effects are expected due to the capsulation of the drug which causes considerable lower plasma levels. Other important aspects of the therapy using liposomal glucocorticoids are the following:

1. Increased permeability of the local vascular endothelium and the presence of activated macrophages facilitate accumulation of liposomes in inflamed areas.
2. Very high glucocorticoid concentrations are reached at the site of inflammation for several hours which might allow complete utilization of genomic and non-genomic glucocorticoid actions.

For these reasons, long circulating liposomal glucocorticoids may have an improved benefit-risk ratio. However, we are still waiting for the first results describing the effects in humans.
Nitro-steroids

Another approach to develop improved glucocorticoids represents the so-called nitro-steroids. An aliphatic or aromatic linker molecule associates a glucocorticoid derivate with nitric oxide (NO). NO, which can enhance anti-inflammatory effects of glucocorticoids, is slowly released from these drugs [42]. Two interesting substances are NO-prednisolone (NCX-1015) and NO-hydrocortisone (NCX-1004). It has been shown in murine arthritis models that NCX-1015 caused anti-inflammatory effects 10-fold stronger than conventional prednisolone. At the same time fewer side effects are anticipated on the bone compartment, since elevated bone resorbing activity of rat primary osteoclasts was observed for prednisolone but not for NCX-1015 [42-44]. In order to explain the strong anti-inflammatory effects of nitro-steroids, post-translational modification of GCR through tyrosine nitration is suggested [42]. Further studies are needed to investigate the potential of these drugs in clinical practice.

Selective glucocorticoid receptor agonists (SEGRA’s)

As mentioned before, the genomic actions of GC’s can be divided into two processes: “transactivation”, which is thought to be responsible for most of the adverse reactions [3], and “transrepression”, which is considered to mediate numerous desirable anti-inflammatory and immunomodulating effects [45]. Hence, “dissociating glucocorticoids” or so called “selective glucocorticoid receptor agonists (SEGRA’s)” are in the development pipeline. These drugs cause a receptor conformation preferring GCR/protein interaction rather than GCR/DNA binding. Consequently, the advantage of such innovative steroidal or non-steroidal molecules is that they are capable of inducing transrepression processes whereas induction of transactivation is negligible. The first steroidal compounds (Roussel Uclaf currently Sanofi Aventis, Paris, France), including RU 24782, RU 24858 and RU 40066, were convincing in vitro but unfortunately not in vivo [46,47]. However, in the last years an increasing number of
SEGRA’s has been described. A very interesting substance is for example A276575 and its four enantiomers [45], all ligands that bind with the same affinity to the cGCR as dexamethasone does. These novel GCR ligands repress IL-1α-induced IL-6 production. Importantly, further studies revealed that A276575 and its four enantiomeres showed only less than 5% of the ability of dexamethasone with respect to transactivation properties. Another interesting SEGRA is the substance ZK216348 [5], which also induces potent anti-inflammatory effects, but its capacity to transactivate is 60 times weaker compared to prednisolone and even over 300 times weaker than dexamethasone.

In conclusion, these novel substances may be introduced into clinical medicine in the nearby future, but also for these drugs in vivo studies are obviously needed to define their benefit-risk ratio in humans first. The reason is that the success in animal studies does not ensure efficacy in the complex situation of human disease. Secondly, SEGRA effects observed on the single cell level in vitro may be different from those that occur on different cell types in vivo. Finally, given that some of the beneficial GC effects are mediated via transactivation (i.e. the induction of anti-inflammatory molecules) it can be assumed that SEGRA’s -acting (virtually exclusively) via transrepression- may not reach the overall immunosuppressive potential of conventional GC’s in vivo.
Conclusion

In summary, over the last years our knowledge on glucocorticoids – being the most important and most frequently used anti-inflammatory and immunosuppressive drugs – and their mechanisms of action has increased enormously. The cytosolic glucocorticoid receptor has been shown not only to mediate well known genomic actions, but is also involved in rapid non-genomic effects due to complex interactions with various signalling processes. Other hot spots in glucocorticoid research are the therapeutic relevant discrimination of genomic mechanisms into transactivation and transrepression and – last but not least – the efforts being made to clarify the functional role of membrane-bound glucocorticoid receptors. All of this new information will hopefully soon result in the successful development of new glucocorticoid receptor ligands that improve clinical medicine by demonstrating a better benefit-risk ratio compared with conventional GCs.
Acknowledgement

Daniel Hommes is financially supported by The Netherlands Organization for Health Research and Development. Frank Buttgereit’s work is supported by the DFG (BU 1015/7-1) and by the Federal Ministry of Education and Research (01GS0110/01GS0160/01GS0413).
References


Legends

Figure 1. Genomic mechanisms of GC action: The ligand activated GC receptor translocates into the nucleus where it induces or inhibits gene transcription. (I) Induced synthesis of anti-inflammatory proteins through binding of the ligand-activated glucocorticoid receptor to positive GREs; (II) suppressed transcription of inflammatory genes via negative GREs; (III) “transrepression” through direct or indirect interaction with transcription factors; (IV) competition for nuclear coactivators between the GC/cGCR complex and transcription factors.

Abbreviations: cGCR: cytosolic glucocorticoid receptor; GRE: glucocorticoid response elements; nGRE: negative GRE; AP-1: activator protein 1 [10]

Figure 2. Summary of genomic and non-genomic mechanisms of GC action: Lipophilic glucocorticoids pass easily through the plasma membrane and bind to the cGCR which mediate genomic (I) and non-genomic effects (II). Non-genomic effects are also suggested to be mediated via membrane bound GCR (III) and by interactions with cellular membranes (IV). [10]

Table 1. Glucocorticoid effects on primary and secondary immune cells [2]
Genomic mechanisms

I. cGCR binds to GRE, leading to transcription.

II. cGCR binds to nGRE, inhibiting transcription.

III. p65 and p50 do not bind to kB site, resulting in no transcription.

IV. cGCR binds to AP1 site, preventing transcription.

Figure 1
Figure 2

Genomic mechanisms

Nongenomic mechanisms

- Glucocorticoid
- mGCR-mediated nongenomic m.
- cGCR-mediated genomic m.
- cGCR-mediated nongenomic m.
- mGCR-mediated nongenomic m.
- Nonspecific nongenomic m.

Antiinflammatory, immunomodulatory and other (including unwanted adverse) effects
Table 1

<table>
<thead>
<tr>
<th>Type of Cell</th>
<th>Effect 1</th>
<th>Effect 2</th>
<th>Effect 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monocytes/macrophages</td>
<td>↓ number of circulating cells</td>
<td>↓ myelopoiesis</td>
<td>↓ release</td>
</tr>
<tr>
<td></td>
<td>↓ expression of MHC class II</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>molecules and Fc receptors</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓ synthesis of pro-inflammatory</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>cytokines (eg IL-2, IL-6, TNFa)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>and prostaglandins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T cells</td>
<td>↓ number of circulating cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(redistribution effects)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓ production and action of IL-2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(most important)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Granulocytes</td>
<td>↓ number of eosinophile and</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>basophile granulocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>↑ number of circulating</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>neutrophils</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endothelial cells</td>
<td>↓ vessel permeability</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓ expression of adhesion</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>molecules</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓ production of IL-1 and</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>prostaglandins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>↓ proliferation</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓ production of fibronectin</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>and prostaglandins</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>