What makes a good anti-inflammatory drug target?

David L. Simmons

Inflammation Discovery Research, Wyeth Research, 200 Cambridge Park Drive, Cambridge, MA 02140, USA

This review focuses on the major, 'successful' target families in inflammation and attempts to identify some of the key features of what makes a good anti-inflammatory target. The review is based on a systematic analysis of approved anti-inflammatory drugs grouped according to their drug–target family. The cytokine family is a drug-dense area. They have yielded and continue to yield a rich stream of drugs. As in other therapeutic areas, G-protein-coupled receptors (GPCRs), also known as seven-transmembrane pass receptors, have provided significant drug targets. In addition, the superfamilies of cell adhesion molecules and co-stimulatory molecules, which have special relevance to immune processes, have begun to provide the first approved drugs and might yield many more. The recent, rapid increase in the number of defined targets in the immune system – leukocyte surface antigens, cytokines, GPCRs, adhesion molecules and co-stimulatory molecules – will ensure a rich stream of future anti-inflammatory drug targets.

Inflammation is the body's way of dealing with infections and tissue damage, but there is a fine balance between the beneficial effects of inflammation cascades and their potential for long-term tissue destruction. If they are not controlled or resolved, inflammation cascades can lead to the development of diseases such as chronic asthma, rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease and psoriasis. Within many inflammation cascades or pathways there are often pivotal molecular targets that, when antagonized or neutralized, block the output of the pathway. Historically, at least over the past 20 years in the modern era of target-based drug discovery, a relatively small number of pivotal targets have been identified that have yielded any successful anti-inflammatory drugs. Most of these are antagonists of endogenous proinflammatory mediators such as prostaglandins, leukotrienes and histamine. These targets include the H1 receptor for histamine, the enzymes cyclooxygenase 1 and 2 (COX-1 and COX-2), the cytokine tumor necrosis factor-α (TNF-α) and the receptor for the cysteinyl leukotrienes C4 and D4. The physiological anti-inflammatory properties of glucocorticoids have been extensively exploited in many inflammatory and autoimmune diseases (Table 1 and Table 2) [1]. Two conclusions are possible – first, that the field of inflammation only has a small number of pivotal regulatory targets or, second, that it reflects the relative lack of knowledge of the immune system 20 years ago (and hence the paucity of candidates that could be exploited at that time).

Over the past 20 years there has been a significant increase in knowledge about immunology, both in terms of molecular targets and molecular mechanisms. For example, 339 leukocyte surface antigens have been characterized [cluster of differentiation molecules (CD)1–339 (HLDA8)] (www.HLDA8.org), >80 cytokines and their receptors have been identified [2], >20 chemokines and their G-protein-coupled receptors (GPCRs) defined and, in addition to these, >50 other GPCRs with potential roles in inflammation have been described [3]. This has led to a recent increase in the number of targets being pursued in discovery and clinical development.

Out of all of these new molecules (leukocyte surface antigens, cytokines, chemokines and GPCRs) what makes a good anti-inflammatory target? Using an analysis of historical data and of currently marketed drugs, the aim of this review is to establish some basic principles that guide us to the answer to this question.

What makes a good anti-inflammatory target? – The basic principles

There are some basic principles that guide the discovery and development of successful therapeutic targets. First, the target might be proximal to the initiation of the disease – not necessarily the

Corresponding author: Simmons, D.L. (dsimmons@wyeth.com).

1359-6446/06/$ – see front matter PII: S1359-6446(05)03721-9

TABLE 1

Major anti-inflammatory targets (by class)

Target class	Specific targets	Examples of approved drugs
Enzymes	COX-2	Celebrex®, Arcoxia®
	COX-1 and COX-2	Voltaren®, NSAIDs
	IMPDH	Cellcept®
G-protein-coupled receptors	CysLT1	Singulair®, Accolate®
	H1	Zyrtec®, Clarinex®
Nuclear hormone receptors	Corticosteroids	Flonase®, Flixonase®, Nasonex®
Cytokines and cytokine receptors	TNF-α and TNF-RII	Remicade®, Enbrel®, Humira®
	IL-1β and IL-1RA	Kinneret®
	IL-2 and IL-2R	Zanapex®, Simulect®
	Interferon α2	Pegintron®, Pegasys®
	Interferon β1	Avonex®, Rebif® Betaseron®
	Interferon γ	Actimmune®
Cell interaction molecules (cell adhesion molecules and co-stimulatory molecules)	LFA-1 and CD11a	Raptiva®
	CD2 and LFA-3	Amevive®
	VLA-4 and CD49d	Tysabri® (appproved 2004, withdrawn February 2005 and re-submitted November 2005, currently under review)
	CTLA-4-Ig	Orencia™

This table lists all the major anti-inflammatory targets organized according to the class of target (enzymes, G-protein-coupled receptors, nuclear hormone receptors, cytokines, and cell adhesion molecules and co-stimulatory molecules) with specific targets and examples of approved drugs.

Abbreviations: COX, cyclooxygenase; IMPDH, inosine monophosphate dehydrogenase; NSAIDs, nonsteroidal anti-inflammatory drugs; CysLT1, cysteinyl leukotriene 1; H1, histamine 1; TNF-α, tumor necrosis factor-α; TNF-RII, tumor necrosis factor-α receptor II; IL, interleukin; IL-2R, interleukin 2 receptor; IL-1RA, interleukin 1 receptor antagonist; LFA-1, leukocyte function -associated antigen-1; VLA-4, very late activation antigen 4; LFA-3, leukocyte function-associated antigen 3; CTLA-4Ig, cytotoxic T lymphocyte antigen 4 immunoglobulin chimera.

very first initiating event but at least close to it. Second, the target could play a pivotal or driving role in the disease process – these targets are often at key regulatory points or rate-limiting steps in pathways (so that blocking such an event stops a whole series of downstream processes). Third, targets could be unique to the disease process – thus giving a desired potent effect on the disease without having unwanted side effects on other physiological processes. Not all of these features need to be present to ensure success but at least one is desirable for an effective drug target (and this is also true in the field of anti-inflammatory drug discovery).

There are some specific features of immune responses and inflammatory cascades that can be exploited to yield good anti-inflammatory targets. Leukocytes are highly mobile cells constantly moving from the vascular space to the tissue space and back, via the lymphatic system, into the blood system. They use a unique set of adhesion molecules and chemoattractant molecules to guide this migration process. They also have a highly specific and distinctive signaling system initiated by the primary recognition of specific antigens (by either B cell receptors or T cell receptors) and distinctive secondary signaling systems to control and modulate those primary signals. They also use cytokines as systemic and more-localized or paracrine communicators. Many powerful proinflammatory mediators, such as histamine and leukotrienes, are released early in inflammation cascades and blocking their actions has proven to be a successful source of anti-inflammatory drug targets.

In the era of high-throughput biology (from sequencing to screening) it is hoped that targets can de identified, validated and translated into clinical candidates in ever decreasing periods of time [4]. However, it should be acknowledged that it often takes many years to understand fully the biology of a particular target and to select the best utility of this in particular diseases.

A key example in the field of inflammation is TNF-α. In the early 1970s, TNF-α was first defined as a potent muscle-wasting (or cachexic) factor with potent antitumor activity [5,6]. This initially led to its development as a therapy for cancer [7] in the mid 1980s. However, it proved to be too toxic or, in fact, made cancers worse. The TNF-α field then turned to the opposite approach – inhibition of TNF-α with neutralizing antibodies or TNF-α receptor chimeric fusion proteins – initially in the field of sepsis, where anti-TNF-α neutralizing antibodies were either ineffective or made survival outcomes worse [6]. These results could have been potentially terminal to the whole field of anti-TNF-α therapies but people persevered and, in the early 1990s, a pioneering clinical trial in rheumatoid arthritis demonstrated, for the first time, the beneficial effects of blocking TNF-α in autoimmune disease [8,9]. This subsequently lead to several approved therapies, either monoclonal antibodies blocking TNF-α (Remicade® and Humira®) or a recombinant TNF-α -receptor-II chimeric fusion protein (Enbrel®). Utility for some of these agents has also been demonstrated in ankylosing spondylitis, psoriatic arthritis, psoriasis and inflammatory bowel disease. Figure 1 shows that a single class of anti-inflammatory therapeutics, the anti-TNF-α biologics, has the potential to be effective in multiple diseases [9].

Thus, it took 30 years to move from the identification and purification of TNF-α to the successful development of therapeutics. Of course, scientific and technological progress has accelerated over the past two decades. However, it is highly probable that it will take several attempts to define the potential of some of the more recent targets.

In the following paragraphs, I will analyze the major, 'successful' target classes in inflammation. Success here is strictly defined by marketed drugs – it discounts target classes, such as kinases, for which

TABLE 2

Best selling anti-inflammatory drugs

Target and drug class	Disease	Drug or brand name	2004 sales (US$ million)
COX-2 inhibitors	Pain associated with osteoarthritis and rheumatoid arthritis	Celebrex®	3302
		Bextra®	1286
		Vioxx®	1489
COX-1 and COX-2 NSAIDs	Pain associated with osteoarthritis and rheumatoid arthritis	Voltaren®	638
		Mobic®	836
Leukotriene (CysLTB4) receptor antagonists	Asthma	Singulair®	2622
		Accolate®	116
Steroids (inhaled)	Rhinitis	Flonase®–Flixonase®	1058
		Nasonex®	594
		Rhinocort®	361
Steroids (inhaled)	Asthma	Flovent®–Flixotide®	1131
		Pulmicort®	1050
Steroids (inhaled in combination with β agonists)	Asthma	Advair®–Seretide®	4503
		Symbicort®	797
H1 antagonists	Rhinitis	Zyrtec®	1287
		Clarinex®–Aerius®	692
		Allegra®–Telfast®	1868
IMPDH inhibitor	Transplant rejection	Cellcept®	1129
FKBP12–Calcineurin inhibitor	Transplant rejection	Prograf®	985
Anti-CD25 and IL-2R p55 antibody	Transplant rejection	Zenapax®	32
		Simulect®	
Immunosuppressive peptide. Cyclosporin	Transplant rejection	Neoral®–Sandimmune®	1011
Anti-TNF-α biologics	Rhematoid arthritis, psoriasis, ankylosing spondylitis, Crohn's disease	Remicade®	2891
		Enbrel®	2580
		Humira®	852
CD2 antagonist LFA3-Fc	Psoriasis	Amevive™	43
Anti-LFA-1–CD11a antibody	Psoriasis	Raptiva®	56
α-interferons (IFN-α2)	Hepatitis C	Pegasys®	949
		Pegintron®	563
β-interferons (IFN-β1)	Multiple sclerosis	Rebif®	1091
		Avonex®	1420
		Betaseron®–Betaferon®	973
Immunosuppressant synthetic peptide co-polymer	Multiple sclerosis	Copaxone®	936

Not all anti-TNF-α biologics are approved for all the diseases indicated. This table lists the best selling anti-inflammatory drugs in 2004 organized according to the target class, the major diseases they are approved to treat, the major market drug brand name and the 2004 total sales in US$ million.

Abbreviations: COX, cyclooxygenase; NSAIDs, nonsteroidal anti-inflammatory drugs; CysLTB4, cysteinyl luekotriene B4; H1, histamine 1; IMPDH, inosinemonophosphate dehydrogenase; FKBP12, FK-binding protein 12; IL-2R, interleukin 2 receptor; TNF- α, tumor necrosis factor- α; IFN, interferon; LFA-3: leukocyte function-associated antigen 3; LFA-1, leukocyte function associated antigen 1.

there are currently no approved anti-inflammatory drugs. However, I am not suggesting that we ignore kinases as potential targets.

In inflammation the cytokine family is a drug-dense area, in fact a basic premise of this review is that cytokines have yielded a rich stream of drugs and are the 'GPCRs of immunology'. There are two additional families of molecules involved in cell–cell interactions, the adhesion molecules and the co-stimulatory molecules (CSMs). They have special relevance to immune processes that have also yielded approved drugs and could yield many more.

I will now discuss the analysis of historical data regarding the known cytokines, GPCRs and cell interaction molecules that underpin this conclusion.

Cytokines as drugs and drug targets

Cytokines are a large family of proteins, comprising ~93 members and ~96 receptors that are key signaling mediators in immune systems. Figure 2 shows the 'total known cytokine world' and how it is divided into distinct subfamilies. The term cytokine is reasonably well-defined – soluble proteins produced by leukocytes or other cell types that act as chemical communicators between cells. In some cases the term can encompass growth factors, such as epidermal growth factor, transforming growth factors and nerve growth factors, but here I will restrict the term to cytokines that are clearly linked to the immune system either by major site of origin or major site of action.

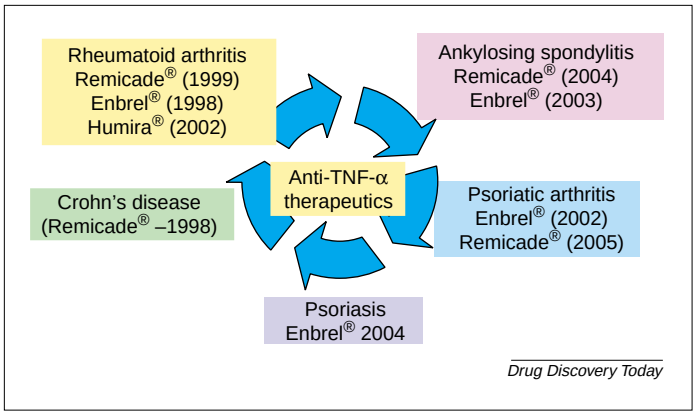

FIGURE 1

Anti-inflammatory therapeutics have the potential to be effective in multiple diseases. This figure illustrates that single therapeutics (here, exemplified by biological therapeutics specifically targeting tumor necrosis factor-α) can have efficacy in multiple autoimmune diseases. The agents, all of which neutralize TNF-α, are Enbrel® (a fusion protein of TNF-α receptor type II and human immunoglobulin (Ig)-Fc), Remicade® and Humira® (two monoclonal antibodies). The diseases they have been approved for, and the dates of the approvals in the US, are listed.

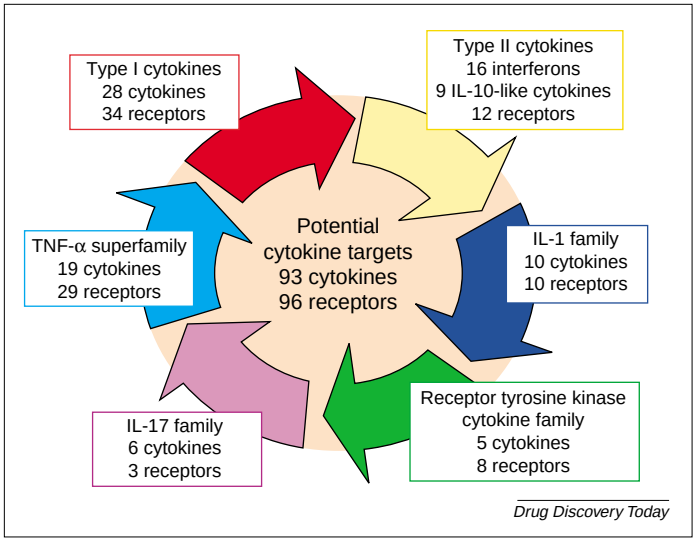

FIGURE 2

Potential cytokine targets. This figure illustrates the pool of all potential cytokines and their receptors that are currently known to exist. With the complete sequence of the human genome now known, it is probable that there will only be a small number of additional members of this extended family. To date, 6% of this family has already yielded approved anti-inflammatory therapeutics and, hence, is a rich source of potential future drug targets.

Unfortunately, the cytokine nomenclature is not based on any systematic relationships between the molecules; it reflects the different ways they were first discovered. However, a consensus has emerged based on classifying the receptors for cytokines. Receptor subfamilies are based on common structural features defined by either extensive primary sequence homologies or more-restricted structural motifs. The main subfamilies of cytokine receptors are: the haematopoietin receptors (also termed the type I receptors), which include many of the interleukins; the interferon receptors (also termed the type II receptors); the IL-1 and toll-like receptors; the tyrosine kinase receptors; and the TNF-α receptors.

In total, there are 28 haematopoietin (type I) receptor cytokines (and 34 receptors) and 25 interferon (type II) cytokines (and 12 receptors) [10,11]. The type II receptor cytokines include the interferons (16 genes encoding α and β interferons plus a single γ gene) [12] and the IL-10 family (IL-10 plus eight IL-10-related cytokines) [13,14]. The IL-1–toll-like receptor subfamily consists of ten cytokines and ten receptors [15–18], the receptor tyrosine kinase family consists of five cytokines and eight receptors, and the TNF-α superfamily consists of 19 TNF-α cytokine homologues and 29 receptors [19–21].

A potential, new subfamily might be based on IL-17 and related proteins; to date it consists of six members, all are structurally distinct from type I and II cytokines [22]. Receptors for this family are still being assigned and identified but there are three known to date.

Potentially, all of the cytokines can be exploited as therapeutics directly, or neutralized by antagonist agents such as monoclonal antibodies or receptor chimeric fusion proteins. Cytokine receptors can also be targeted with neutralizing antibodies.

To date, there are nine approved cytokine-based therapeutics. The nine cytokines or their receptors have yielded 16 approved drugs (with approval in at least one major G8 country). Table 3 lists all the approved drugs based on cytokines or their receptors, therefore 6% of this family of proteins has yielded clinically validated targets. This is, of course, a snapshot today, reflecting the early era of cytokine discovery in the 1970s and 1980s, because it takes at least 10–15 years from initial discovery to approved therapy. Moving forward, this number will increase, especially in relation to the use of receptors as targets and as therapeutics. Often, the cytokine receptor is identified some time after the discovery of the cytokine so there is an extra lag time in understanding the biology of the receptor and hence exploiting its potential as a target.

A successful track record in inflammation currently makes the cytokine family of proteins the equivalent of the GPCR family in the cardiovascular and neuroscience fields, it is a rich hunting ground for drug candidates. Thus, the cytokine family is a unique feature of inflammation drug discovery.

One major shift in the field has been towards the increasing use of antagonism of the action of cytokines rather than using of the cytokine itself as a therapeutic. Early on, it was hoped that many of the cytokines themselves would be drugs (i.e. direct administration of the cytokine to patients would treat the disease). This concept is true for interferons α2, β1 and γ but, so far, not for any of the other family members – at least not in inflammatory diseases. Outside the field of immunology there have been highly successful examples of the direct use of cytokines, including erythropoietin and granulocyte-colony stimulating factor (G-CSF), in the fields of oncology and renal disease.

Clinical trials involving direct administration of some of the more recently discovered cytokines, such as IL-18, IL-20 and IL-21, are certainly still being pursued, but often in oncology to stimulate an antitumor immune response. However, therapeutics based on neutralizing these cytokines or antagonizing their receptor function could yield additional drugs with anti-inflammatory effects.

Many cytokines (but by no means all) are proinflammatory and directly contribute to the development of inflammation and chronic autoimmune disease pathologies. Antagonizing or neutralizing their actions is now the preferred method of targeting the

TABLE 3

Cytokine-based therapeutics (approved drugs)

Cytokine	Therapeutic	Disease
Interferon α2a	Intron® A	Hepatitis B, hepatitis C
	Roferon®	
	Pegintron®	
	Pegasys®	
Interferon β1	Rebif®	Multiple sclerosis
	Avonex®	
	Betaseron®	
Interferon γ	Actimmune®	Chronic granulomatous disease
TNF-α (antagonists)	Remicade®	Rheumatoid arthritis, Crohn's disease, ankylosing spondylitis, Psoriatic arthritis
	Enbrel®	Rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, juvenile arthritis, psoriasis
	Humira®	Rheumatoid arthritis
IL-1β (antagonist)	Kineret®	Rheumatoid arthritis
IL-2 (antagonist)	Zenapax®	Transplantation
IL-2R (receptor antagonist)	Simulect®	Transplantation
IL-6R (receptor antagonist)	Actemera® (MRA–IL-6R mab)	Castleman's disease (approved in Japan, June 2005)
IL-11	Neumega	Chemotherapy-induced thrombocytopenia

These are all the approved drugs based on either direct use of the cytokine as a drug, or antagonists of the cytokine or its receptor. The cytokine is listed on the left, then brand names of the marketed therapeutics in the center and the major (although not all) diseases the drugs are approved to treat are listed on the right. Pegintron® and Pegasys® are pegylated versions of interferon α2a.

Abbreviations: TNF-α, tumor necrosis factor-α; IL, interleukin; IL-2R, interleukin 2 receptor; IL-6R, IL-6 receptor.

disease. However, there are also some anti-inflammatory cytokines, such as IL-10, that have been administered directly to reduce inflammation but, so far, without success.

The potential of cytokines and their receptors as drug targets is clearly established. What is not so clear is which cytokines are the key players in specific diseases, whether some are pivotal regulators of cytokine networks or whether they act in a more restricted fashion and, finally, whether redundancy in the actions of many cytokines will limit the efficacy of neutralizing single agents. Even for the well-established case of TNF-α, we do not know whether the clinical efficacy seen is caused by the fact that TNF-α is a major regulator of other cytokine networks and hence yields a powerful multicytokine effect, or whether TNF-α is just a strong driver of the specific diseases it is involved in.

Interleukins as drugs and drug targets

We can get a more detailed measure of the success and/or failure rates of cytokines by analyzing one of the subfamilies, the interleukins. Interleukins are cytokines that are largely, but not exclusively, produced by T cells, which are key cells involved in initiating and controlling immune responses. Hence, the T-cell-derived cytokines are likely to be pivotally important in inflammation. Interleukin (IL) numbers (e.g. IL-1) have been assigned in chronological order of discovery. It is important to state here that, although the term interleukin is well-established and still widely used, they are a very heterogeneous group of proteins, both structurally and functionally. The value in focusing on the interleukins is that this group, especially the first members to be discovered, has had more time to progress through clinical development and, hence, to yield data for analysis.

Table 4 lists the first 20 interleukins (IL-1–IL-20) and their track record of yielding drugs and potential drug candidates. The first ten interleukins discovered (IL-1–IL-10) contain three validated targets (IL-1, IL-2 and IL-6) and four approved drugs in total, two of which are based on IL-2. For the group of interleukins from IL-11 to IL-20 there is, so far, one validated target, IL-11. Because many from this group, IL-11–IL-20, were only discovered in the past 5–15 years, a lot of them are still in clinical trials (at proof of concept stage). Some of the interleukins in this group, such as IL-12, IL-13, IL-15 and IL-18, show great promise as targets and emerging data indicates drug activity in clinical trials [23,24]. It is, therefore, possible that this group will yield the same number of drug candidates as the first series (3 out of 10). For the group containing IL-21–IL-32, it is too early to say whether they contain any promising drug targets [25–27].

It is still possible that the full potential of even the 'early' interleukins has not yet been established. For example, anti-IL-1β antibodies are in clinical development (www.amgen.com) and could prove to be superior to the approved agent, anakinra (Kineret®), which is based on the IL-1 receptor (IL-1R) antagonist (IL-1RA). In addition, the partial efficacy seen with anti-IL-4 and anti-IL-5 antibodies might reflect suboptimal dosing or design of clinical trials. Thus, to date, IL-4 and IL-5 have not been fully clinically validated and have not yielded approved therapeutics.

The overall conclusion here is that cytokines have produced an excellent track record of successful drugs. This justifies the view that cytokines have high drug-density potential and supports a continued intensive focus on the more recently discovered family members.

Adhesion molecules and CSMs

Adhesion molecules and CSMs play distinctive roles in inflammatory processes. Cell adhesion molecules (CAMs) are key players in mediating leukocyte migration from the vasculature blood stream

TABLE 4

Interleukins – drugs and potential drug candidates

Interleukin	Disease	Approved therapeutic
IL-1β (antagonist)	Autoimmune, rheumatoid arthritis	Kineret®, IL-1R Antagonist
IL-2 (antagonist)	Transplantation	Zenapax®, anti-IL-2 in transplantation
IL-2 receptor (antagonist)		Simulect®, anti-IL-2R p55 in transplantation
IL-3	Haematopoietic growth factor, not useful as an anti-inflammatory	Not effective in clinical trials
IL-4 (antagonist)	Anti-IL-4 and IL-4R, partial efficacy in asthma	None approved
IL-5 (antagonist)	Anti-IL-5, partial efficacy in asthma	None approved
IL-6 (IL-6 receptor antagonist)	Rheumatoid arthritis	Actemera®, anti-IL-6 receptor in late-stage trials in rheumatoid arthritis in US and Europe, approved in Japan for Castleman's disease
IL-7	No defined disease association to date	None approved
IL-8	Acute infection	None approved
IL-9	No defined disease association to date	None approved
IL-10	Autoimmune – IL-10 is a direct negative regulator of immune responses	None approved, poor pharmacokinetic properties
IL-11	Haematopoietic growth factor, not useful as an anti-inflammatory	Neumega®, approved as a platelet stimulator in chemotherapy
IL-12 (antagonist)	Autoimmune disease	Anti-IL-12 is in Phase II clinical trials for multiple sclerosis and Crohn's disease, early data indicate drug activity IL-12 is in clinical trials as a direct vaccine adjuvant IL-12 was used as an immunostimulant in cancer therapy without success
IL-13 (antagonist)	Asthma, fibrosis	Anti-IL-13 in Phase I and II clinical trials for asthma
IL-14	No defined disease association to date	None approved
IL-15 (antagonist)	Autoimmune disease, rheumatoid arthritis	Anti-IL-15 in Phase II clinical trials for rheumatoid arthritis
IL-16	No defined disease association to date	None approved
IL-17 (antagonist)	Autoimmune disease	Preclinical work only
IL-18 (antagonist for autoimmune disease, agonist for cancer)	Autoimmune disease and cancer	IL-18 is in clinical trials for cancer therapy. Anti-IL-18 is in clinical trials for rheumatoid arthritis
IL-19	No defined disease association to date	None approved
IL-20	Oncology	IL-20 is in clinical trials for cancer therapy

These are all the approved interleukin based drugs, involving either direct use of an interleukin as a drug or the administration of antagonists of an interleukin or its receptor. The interleukin is listed on the left – if the therapeutic is an antagonist it is indicated after the interleukin. The major diseases they are either approved to treat or have been investigated in clinical trials are listed in the center. The right-hand column lists examples of either marketed therapeutics or comments on the current state of clinical trials or approvals.

IL-3 is a haematopoietic multipotential colony-stimulating factor, not an anti-inflammatory agent. IL-8 is a chemokine that binds to a distinct G-protein-coupled receptor. IL-11 has anti-inflammatory activity, but its approved use is as a therapy in oncology to stimulate platelet development in patients undergoing chemotherapy. IL-14 data are not fully supportive of robust cytokine activity. IL-16 data are not fully supportive of robust cytokine activity.

Abbreviations: TNF-α, tumor necrosis factor-α; IL, interleukin.

to sites of inflammation and then leukocyte re-circulation to the lymphatic system and lymphoid organs. CAMs form specific pairings of receptors and ligands expressed on adjacent cells, leading to either transient or sustained intercellular adhesion. In addition, some CAMs link cells to the extracellular matrix of proteins.

Drugs that block specific adhesion events have the potential to be good anti-inflammatory targets because they satisfy several key criteria. Adhesion events are often early in the process (e.g. the primary influx of leukocytes at sites of inflammation is an early event). Adhesion events are also pivotal, if leukocytes cannot enter a site all downstream processes are stopped. Additionally, adhesion events are specific and they involve molecules that are only expressed on leukocytes or vascular endothelium.

CSMs deliver essential signals to leukocytes (especially T cells) to generate and sustain immune responses. T cells recognize specific antigens expressed on MHC molecules, delivered to them by antigen-presenting cells. T cells express a diverse repertoire of T cell receptors (specific for different antigens) and when an antigen is recognized a signal is delivered to the T cell. However, alone, this primary signal is insufficient to yield a positive response unless a simultaneous secondary signal is delivered via the engagement of CSMs expressed on the T cell and the antigen-presenting cell. Like CAMs, the CSMs form distinctive receptor–ligand pairings on the closely apposed T cell and antigen-presenting cell. Thus, CSMs also represent potentially good anti-inflammatory targets. Co-stimulatory events occur early in the initiation phases of immune responses, they are pivotal because blocking them prevents subsequent downstream activation and they are specific to leukocytes.

Although CAMs and CSMs have different functional effects on leukocytes they share the common feature of requiring close cell–cell interaction to mediate their effects, so I have considered them together as drug targets – the cell interaction molecules.

Because one of the guiding principles of good drug targets is specificity for the disease process, I have restricted the analysis to family members either expressed by leukocytes or involved in leukocyte adhesion. This excludes many of the integrins that are involved in general cell–cell and cell–matrix adhesion, most of the cadherins and, also, immunoglobulin superfamily (IgSF) members that have no known leukocyte function. Applying this filter, there are 53 members of the CAM and CSM superfamilies (Figure 3). Not all of them have been clinically validated, but they represent a pool of potential future targets.

The leukocyte CAMs comprise several distinct families. The three major families are the selectins, the integrins and the IgSF members with leukocyte function. There are three selectins, E-selectin, P-selectin and L-selectin, whereas the integrins are a large family of heterodimeric proteins comprising one α and one β chain, involved in cell–cell and cell–matrix adhesion. The integrins are classified according to their β chain type. There are eight leukocyte-specific integrins, the β1 integrin [very late activation antigen 4 (VLA-4, α4β1)], the β4 integrins (α4β7 and αEβ7), the β2 integrins [leukocyte function-associated antigen-1 (LFA-1, αLβ2), Mac-1 (αMβ2), p150 (αXβ2) and p95 (αDβ2)] and the β3 integrin (αVβ3). The IgSF members share domains that are homologous to the basic domains found in Igs. There are at least 20 leukocyte IgSF members, including CD2, CD166, CD22, CD33, CD96, the CD66 family, intercellular adhesion molecule –1 (ICAM-1, also called CD54), ICAM-2 (CD102), ICAM-3 (CD50), ICAM-4 (CD242), ICAM-5, junctional adhesion molecules 1–3 (JAMs 1–3), L1 (CD171), mucosal addressin CAM-1 (MadCAM-1), MUC18 (CD146), sialoadhesin (CD169) and Vascular Cell Adhesion Molecule-1 (VCAM-1, CD106).

Finally, there are several CAMs belonging to different families that are known as non-IgSF CAMs. There are at least 20 non-IgSF members with various structures: CD39; vascular adhesion protein-1 (VAP-1); E-selectin ligand (ESL); P-selectin glycoprotein ligand-1 (PSGL-1); heat-stable antigen (HSA); glycosylation-dependent cell adhesion molecule 1 (GlyCAM-1); CD44; lymphatic vessel endothelial hyaluronic acid receptor (LYVE)-1; CD6; CD36; CD23; galectin 3; CD34; CD43; CD164; podocalyxin-like protein 1 (PCLP1); CD57; CD98; and 2 cadherins (E and VE) [28–30].

The CSM family is a smaller and more closely related family than the disparate set of CAMs. All CSMs are members of the IgSF, containing either one or two Ig-related domains. There are 12 members of the CSM family, which also comprises five CD28-related members – containing one Ig-variable-related domain [CD28, cytotoxic T lymphocyte antigen-4 (CTLA-4), inducible co-stimulatory molecule (ICOS), programmed death receptor-1 (PD-1) and B- and T-lymphocyte attenuator (BTLA)], and there are seven B7-related members – containing two Ig-related domains [B7.1 (CD80), B7.2 (CD86), inducible co-stimulatory molecule ligand (ICOSL), programmed death receptor-ligand 1 (PD-L1), programmed death receptor-ligand 2 (PD-L2), B7–H3 and B7–H4] [31,32].

Given the key roles CAMs and CSMs play in leukocyte function, their combined families are a potential, rich source of anti-inflammatory targets (Table 5). To date they have yielded four approved drugs: Amevive™, which is a leukocyte function associated antigen-3-Fc (LFA-3-Fc) fusion protein; Raptiva®, which is an anti-leukocyte function associated antigen-1α integrin monoclonal antibody (anti-CD11a); Tysabri®, which is an anti-very late activation antigen-4-α4 integrin monoclonal antibody (anti-CD49a), approved in 2004, withdrawn in 2005 but after an extensive safety review data was resubmitted for restricted approval; and Orincia™, a cytotoxic T lymphocyte antigen-4-Fc (CTLA-4-Fc).

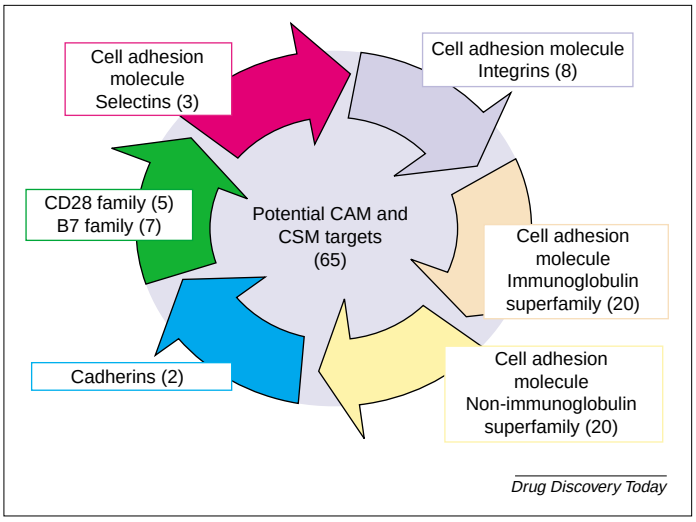

FIGURE 3

Potential cell adhesion molecule and co-stimulatory molecule targets. This figure illustrates the pool of potential cell adhesion molecule (CAM) and co-stimulatory molecule (CSM) targets. This group of targets contains cell surface molecules with diverse structures but they are defined by the functions they possess – either mediating cell–cell or cell–matrix adhesion, or delivering essential signals into immune cells to modulate immune responses. Although this family is smaller than the cytokines, 6% has already yielded clinically validated targets.

Outside the field of inflammation, in the cardiovascular arena the adhesion family has yielded three additional drugs for the treatment of acute thrombotic events following angioplastic interventions. All three drugs are directed at antagonizing the platelet integrin adhesion molecule (gpIIbIIIa). The drugs are ReoPro®, which is a monoclonal antibody fragment (anti-gpIIbIIIa), and two small-molecule drugs, Aggrastat® and Integrilin®.

Thus, from a smaller family base, the yield of validated targets (6%) and approved drugs is comparable with the track record for cytokines. Again, this justifies continued mining of this family for new drug candidates.

GPCRs

GPCRs are integral membrane proteins characterized by having seven trans-membrane domains and also because they engage intracellular G proteins to initiate signaling cascades, upon ligand binding.

In many diseases they are a drug-dense family, particularly in the neuroscience and cardiovascular fields. Surprisingly, in the field of inflammation, GPCRs have yielded a relatively small number of validated drug targets. However, these targets have produced multiple, individual approved drugs that have produced significant medical and commercial value. Two GPCRs, the histamine H1 receptor and the cysteinyl leukotriene 1 (CysLT-1) receptor, are being successfully exploited as targets. The H1 receptor is targeted by three approved drugs for allergic rhinitis (Clarinex®, Allegra® and Zyrtec®), as well as multiple over-the-counter nonprescription products. The CysLT-1 receptor is targeted by three approved drugs for asthma (Singulair®, Accolate® and Onon®).

TABLE 5

Cell interaction molecules: cell adhesion molecules and co-stimulatory molecules – drugs and potential drug candidates

Cell interaction molecule	Disease	Therapeutic
CD11a – leukocyte integrin αL	Autoimmune diseases	Anti-CD11a antibody, Raptiva® approved for psoriasis
CD18 – leukocyte integrin β2	Sepsis, stroke	None approved, either ineffective or exacerbated outcome
ICAM-1	Transplantation, rheumatoid arthritis, myocardial Infarction, burns	Ineffective, murine antibody had severe anti-antibody responses exacerbating disease
		Antisense, in trials for ulcerative colitis
E-selectin	Reperfusion injury	Ineffective
P-selectin	Reperfusion injury	Ineffective
PSGL-Ig, PSGL small-molecule antagonist	Thrombosis	In trials
Platelet integrin gpIIbIIIa	Prevention of thrombotic events in acute cardiovascular interventions	ReoPro®
		Aggrastat®
		Integillin®
Integrin αvβ3	Angiogenesis in cancer, macular disease	In trials, limited efficacy to date
Integrin α4β1	Autoimmune diseases: multiple sclerosis, rheumatoid arthritis, Crohn's disease	Tysabri®, effective but withdrawn because of safety
		Small-molecule programs on hold
		Antisense programs on hold
Integrin α4β7	Autoimmune diseases: multiple sclerosis, rheumatoid arthritis, Crohn's disease	Phase II
CD2	Autoimmune disease: psoriasis	LFA-3-Fc. Amevive™ approved for psoriasis
B7.1 (CD80) and B7.2 (CD86)	Rheumatoid arthritis	CTLA4-Ig. Orencia™

This table lists all the approved drugs targeting cell adhesion molecules or co-stimulatory molecules – here collectively called cell interaction molecules. The cell interaction molecule target is listed on the right followed by the major diseases they are either approved to treat or have been investigated in clinical trials. The last column lists example of either marketed therapeutics or comments on the current state of clinical trials or approvals.

Abbreviations: ICAM, inter-cellular adhesion molecule 1; PSGL-Ig, P-selectin glycoprotein ligand 1-immunoglobulin Fc chimera; gp, glycoprotein; LFA-3, leukocyte function associated antigen 3; CTLA-4Ig, cytotoxic T-lymphocyte antigen 4-immunoglobulin chimera.

There are 750 GPCRs in the human genome, approximately half are olfactory chemosensory receptors and the remaining 367 have natural, endogenous ligands [3]. These GPCRs have been grouped into three large subfamilies – the class A, B and C receptors. Of these, 284 are class A, a family that has classically yielded most drug targets and 73 of these can be identified as having potential roles in inflammation. Thus, it is possible that 25% of all class A GPCRs could be targets for anti-inflammatory drugs.

Figure 4 describes the potential GPCR targets in inflammation. The 73 GPCR targets that could have a function in inflammation include 16 members of the eicosanoid family [three eicosanoids, eight prostanoids, one platelet-activating factor (PAF), two leukotrienes and two CysLTs), as well as three anaphylatoxin receptors, three histamine receptors, eight members of the endothelial differentiation gene (EDG), or sphingolipid, family, four adenosine receptors, two cannabinoid receptors, three formyl Met-Leu-Phe receptors, three tachykinin receptors, four protease-activated receptors (PARs) and six members of the purinoreceptor family.

The largest single family of potential GPCR drug targets is the chemokine family, consisting of 21 members. The term chemokine – derived from chemoattractant cytokine – defines the dominant effect they have on leukocytes, namely to direct the migration of leukocytes to specific sites. Chemokines are small, secreted proteins that bind to specific GPCRs and then activate the cells to migrate, in a directed fashion, towards increasing concentrations of the chemokine. This function has been intensively investigated, following the initial discovery of chemokines in the late 1980s, and it has been demonstrated (in many preclinical disease models) in pivotal roles of inflammation. Hence, this group of targets might be good sources of anti-inflammatory drugs; because they are involved early in initiating events (e.g. controlling the accumulation of activated leukocytes at sites of inflammation), because blocking their action will prevent continued migration, because they are specific and because many chemokine receptors are only expressed on leukocytes.

One surprising statistic is that the track record for the success of the GPCR family in inflammation is, so far, surprisingly low. Only two out of the potential inflammation GPCR subset (containing 73 GPCRs, i.e. 3%) have yielded approved drugs. This rate is low compared with the successful record of GPCRs in other disease areas, such as cardiovascular and central nervous system diseases, which have yielded a higher percentage of clinically validated targets.

However, many of the 73 GPCRs, which have the potential of becoming drug targets, have only recently been cloned and characterized, had their ligands identified and their target-based drug discovery initiated [33]. It is worth noting that the discovery of all three marketed drugs (Singulair®, Accolate® and Onon®), that work by antagonizing the action of CysLTs, pre-dated the molecular characterization of the GPCR target (namely the CysLT-1 receptor, which was only cloned in 1999) [34].

Many of the recently defined prostanoid- and eicosanoid-related GPCRs have great potential for becoming attractive targets because the ligands have been implicated in inflammation processes and they are small molecules (i.e. <500 Da). They are highly amenable to being turned into antagonists, either by blocking the binding of the natural ligands or by modulating the receptor so that it is 'pushed' into a partially active (suboptimal) or a fully inactive state (i.e. partial or inverse agonists).

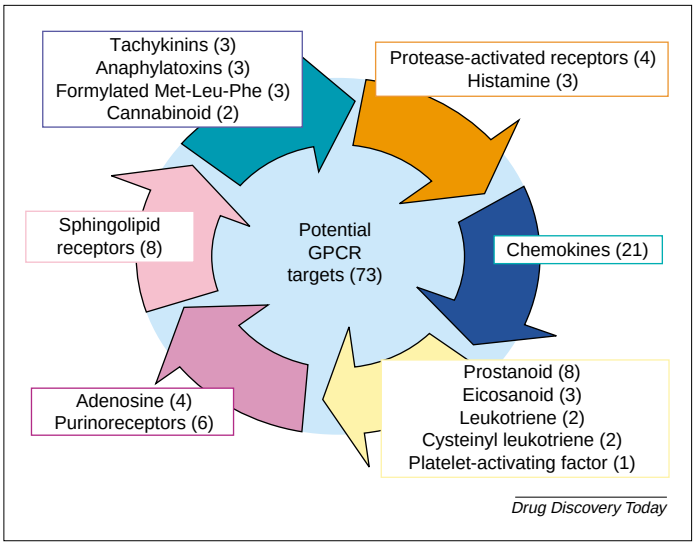

FIGURE 4

Potential G-protein-coupled receptor targets. This figure illustrates the pool of potential G-protein-coupled receptor (GPCR) targets in inflammation. Approximately a quarter of all GPCRs that have natural endogenous ligands in the largest subfamily – the so-called class A subfamily – might play a role in inflammation mechanisms. As GPCRs have proved to yield many approved drug targets in other fields, such as neuroscience and cardio-vascular disease, the hope is that that track record can be repeated in inflammation. To date, however, only 3% of this potential pool has yielded approved therapies.

Full list of potential GPCR targets: The tachykinin family – TACR1, TACR2, TACR3; the sphingolipid family – EDG1, EDG2, EDG3, EDG4, EDG5, EDG6, EDG7, EDG8; the canabinoid family – CNR1, CNR2; the chemokine family – CCR1, CCR2, CCR3, CCR4, CCR5, CCR7, CCR8, CCR9, CCRL1, IL-8RA, IL-8RB, BLR1, GPR2, GPR9, CX3CR1, CCRL2, CCXCR1, CXCR4, CXCR6, CCRL2, CCBP2; the prostanoid family – PTGER1, TBXA2R, PTGFR, PTGER3, PTGER2, PTGDR, PTG1R, PTGER4; the adenosine family – ADORA1, ADORA2A, ADORA2B, ADORA3; the formyl Met-Leu-Phe family – FRRL1, FPR1, FPR2; the anaphylatoxin family – C3AR1, CPR77, C5R1; the leukotriene family – LTB4R2, LTB4R; the platelet activating factor receptor – PTAFR, the cysteinyl leukotriene family – CYSLT1, CYSLT2; the purinoreceptor family – P2RY2, P2RY4, P2RY6, GRR80, P2RY1, GRR91; the protease-activating receptor family – F2R, F2RL1, F2RL2, F2RL3; the eicosanoid family – HM74, GRR81; and the histamine family – HRH1, HRH3, HRH4

The largest subfamily of GPCRs, the chemokine receptors, is currently in the relatively early phase of drug discovery and it is too early to tell how successful targeting these receptors might be. All targets in this subfamily are in early-stage clinical development, yet to progress beyond Phase II.

One notable difference between GPCRs and the chemokine receptors is that the chemokine receptors, although small proteins (5–10 kDa), are larger. Also, most of the current GPCR drug leads are not natural ligands that have been turned into antagonists (although there are a few limited examples of this). Drug candidates will be functional antagonists, such as allosteric modulators, rather than binding antagonists. This will be a key test of the common belief that GPCR targets are particularly amenable to yielding drug candidates, which as been successfully demonstrated in other fields by turning natural ligands into antagonists.

Conclusions

This review has focused on using a combination of guiding principles (that define a good anti-inflammatory target) and an analysis of the approved anti-inflammatory drugs, making some rational statements about the future sources of new candidates.

To recap, the guiding principles that define good anti-inflammatory targets are: first, they are proximal to the initiation of the disease process; second, they are crucial to the driving force of the cascade; and third, they are specific to the inflammation pathway.

By using an analysis of approved anti-inflammatory drugs (broken down by target class) it is possible to make some evidence-based statements about the future potential of exploiting those target classes in the hope of finding a rich stream of new anti-inflammatory drugs.

Inevitably, some areas have not been addressed in this review. Two target classes not considered here are the enzymes and the nuclear hormone receptors (NHRs). Enzymes are a diverse family and it is difficult to identify those specifically involved in inflammation. Enzymes have, of course, yielded the very successful nonsteroidal anti-inflammatory drugs that are based on the inhibition of COX-1 and COX-2. In addition, an inhibitor of inosinemonophosphate dehydrogenase is an effective immunosuppressive drug, used in solid organ transplantation. NHRs have yielded some of the most successful anti-inflammatory drugs, namely corticosteroids, which have been widely exploited in many inflammatory and autoimmune diseases. Although this is the single success to date, out of the 48 known NHRs there is potential for other NHRs, such as the peroxisome proliferator activated receptor-γ (PPAR-γ), according to early preclinical data in some animal models [35].

Another area not considered here, because, as yet, there are no clinically validated candidates, is the potential of exploiting the endogenous anti-inflammation control systems (i.e. investigating lipoxins and annexins) [36]. These systems appear to be the homeostatic controllers of inflammation cascades. They are activated after initial inflammatory events and seek to limit or to resolve that event. Boosting their actions through the development of agonists of (for example) lipoxin, annexin 1 or melanocortin could yield a whole new class of anti-inflammatory drugs [37].

A major conclusion from this review of approved drugs is that cytokines and cell interaction molecules are drug-dense families with a good track record of yielding therapeutics. It fully justifies continued focus on the newer family members.

Over 30 years since the discovery and characterization of the first cytokines, they remain a very attractive area for producing new anti-inflammatory targets, both in terms of direct use of the cytokine or through development of antagonists. To date, the greatest success of the therapeutic use of cytokines has been with biopharmaceuticals – monoclonal antibodies or receptor–Fc fusion proteins.

Some small-molecule agonists and antagonists have proved to be more problematic, but there are some examples in clinical development. There is future potential in this area – seen with the advances in the structural resolution of cytokine–cytokine-receptor complexes that could drive structure-based drug design [38].

The cell interaction molecules also have potential. As for cytokines, all success to date has come from biopharmaceuticals. However, there are several small-molecule modulators in early stages of clinical development that might replicate the efficacy seen with the protein-based therapeutics. One example is the field of the small-molecule antagonists of integrins. Initial research attracted a lot of interest but generated little success. Over the past five years there have been fundamental advances in two areas (high-resolution structural information and greater mechanistic understanding of the mode of action of integrins) [39]. Both these areas might lead to a second phase of drug discovery that could yield success.

The GPCR family has the potential to yield significantly more targets than the best family to date (only 3% of the potential pool of GPCRs has produced approved drugs). Currently, this compares unfavorably with the 6–8% success record seen with cytokines and cell interaction molecules. At least 25% of the largest subfamily of GPCRs (class A) has potential relevance to inflammation and this class has proved to be highly lucrative (in other disease areas) for generating drug candidates. A major near-term test of this potential will be the data coming from clinical trials with chemokine antagonists over the next few years.

Discovery and development of anti-inflammatory drugs has produced effective therapies for several diseases. The analysis in this review identifies some defined areas where we might have the best hope of finding new drugs.

Acknowledgements

I would like to thank Mary Collins at Wyeth for Figure 2.

References

1 Drews, J. and Ryser, S. (1997) Classic drug targets. *Nat. Biotechnol.* 15, 1318–1319
2 Trinchieri, G. (2004) Cytokines and cytokine receptors. *Immunol. Rev.* 202, 5–7
3 Vassilatis, D.K. *et al.* (2003) The G protein-coupled receptor repertoires of human and mouse. *Proc. Natl. Acad. Sci. U. S. A.* 100, 4903–4908
4 Hopkins, A.L. and Groom, C.R. (2002) The druggable genome. *Nat. Rev. Drug Discov.* 1, 727–730
5 Carswell, E.A. *et al.* (1975) An endotoxin-induced serum factor that causes necrosis of tumors. *Proc. Natl. Acad. Sci. U. S. A.* 72, 3666–3670
6 Beutler, B. and Cerami, A. (1988) Tumor necrosis, cachexia, shock, and inflammation: a common mediator. *Annu. Rev. Biochem.* 57, 505–518
7 Jones, A.L. and Selby, P. (1989) Tumour necrosis factor: clinical relevance. *Cancer Surv.* 8, 817–836
8 Elliot, M.J. (1993) Treatment of rheumatoid arthritis with chimeric monoclonal antibodies to tumour necrosis factor alpha. *Arthritis Rheum.* 36, 1681–1690
9 Feldmann, M. and Maini, R.N. (2003) Lasker clinical medical research award. TNF defined as a therapeutic target for rheumatoid arthritis and other autoimmune diseases. *Nat. Med.* 9, 1245–1250
10 Boulay, J.L. *et al.* (2003) Molecular phylogeny within type 1 cytokines and their cognate receptors. *Immunity* 19, 159–163
11 Langer, J.A. *et al.* (2004) The class II cytokine receptor (CRF2) family: overview and patterns of receptor-ligand interactions. *Cytokine Growth Factor Rev.* 15, 33–48
12 Pestka, S. *et al.* (2004) Interferons, interferon-like cytokines and their receptors. *Immunol. Rev.* 202, 8–32
13 Conti, P. *et al.* (2003) IL-10 subfamily members: IL-19, IL-20, IL-22, IL-24 and IL-26. *Immunol. Lett.* 88, 171–174
14 Donnelly, R.P. *et al.* (2004) The expanded family of class II cytokines that share the IL-10 receptor-2 (IL-10R2) chain. *J. Leukoc. Biol.* 76, 314–332
15 Nicklin, M.J.H. *et al.* (2002) A sequence-based map of the nine genes of the human interleukin-1 cluster. *Genomics* 79, 718–725
16 Sims, J.E. (2002) IL-1 and IL-18 receptors, and their extended family. *Curr. Opin. Immunol.* 14, 117–122
17 McInnes, I.B. *et al.* (2001) Interleukin-18: novel cytokine in inflammatory rheumatic disease. *Arthritis Rheum.* 44, 1481–1483
18 Liew, F.Y. *et al.* (2003) Role of interleukin 18 in rheumatoid arthritis. *Ann. Rheum. Dis.* 62 (Suppl. 2), 48–50
19 Moseley, T.A. *et al.* (2003) Interleukin-17 family and IL-17 receptors. *Cytokine Growth Factor Rev.* 14, 155–174
20 Ware, C.F. (2003) The TNF superfamily. *Cytokine Growth Factor Rev.* 14, 181–184
21 Weinberg, A.D. and Montler, R. (2005) Modulation of TNF receptor family members to inhibit autoimmune disease. *Curr. Drug Targets Inflamm. Allergy* 4, 195–203
22 Aggarwal, B.B. (2003) Signalling pathways of the TNF superfamily: a double edged sword. *Nat. Rev. Immunol.* 3, 745–756
23 Watford, W.T. *et al.* (2003) The biology of IL-12:coordinating innate and adaptive immune responses. *Cytokine Growth Factor Rev.* 14, 361–368
24 Gracie, J.A. *et al.* (2003) Interleukin-18. *J. Leukoc. Biol.* 73, 213–224
25 Mehta, D.S. *et al.* (2004) Biology of IL-21 and the IL-21 receptor. *Immunol. Rev.* 202, 84–95
26 Hunter, C.A. *et al.* (2004) The role of IL-27 in the development of T-cell responses during parasitic infections. *Immunol. Rev.* 202, 106–114
27 Hunter, C.A. (2005) New IL-12 family members: IL-23 and IL-27, cytokines with divergent functions. *Nat. Rev. Immunol.* 5, 521–531
28 Yonekawa, K. and Harlan, J.M. (2005) Targeting leukocyte integrins in human disease. *J. Leukoc. Biol.* 77, 129–140
29 Steinman, L. (2005) Blocking adhesion molecules as therapy for multiple sclerosis:natalizumab. *Nat. Rev. Drug Discov.* 4, 510–519
30 Isacke, C.M and Horton M.A. (2000) *The Adhesion Molecules FactsBook*, Academic Press
31 Rudd, C.E. and Schneider, H. (2003) Unifying concepts in CD28, ICOS and CTLA4 co-receptor signaling. *Nat. Rev. Immunol.* 3, 544–556
32 Greenwald, R.J. *et al.* (2005) The B7 family revisited. *Annu. Rev. Immunol.* 23, 515–548
33 Wise, A. *et al.* (2004) The identification of ligands at orphan G-protein coupled receptors. *Annu. Rev. Pharmacol. Toxicol.* 44, 43–66
34 Lynch, K.R. *et al.* (1999) Characterization of the human cysteinyl leukotriene CysLT1 receptor. *Nature* 399, 789–793
35 Gronemeyer, H. *et al.* (2004) Principles for modulation of the nuclear receptor superfamily. *Nat. Rev. Drug Discov.* 3, 950–964
36 Gilroy, D.W. and Peretti, M. (2005) Aspirin and steroids: new mechanistic findings and avenues for drug discovery. *Curr. Opin. Pharmacol.* 5, 405–411
37 Gilroy, D.W. *et al.* (2004) Inflammatory resolution: new opportunities for drug discovery. *Nat. Rev. Drug Discov.* 3, 401–416
38 Rickert, M. *et al.* (2005) The structure of interleukin-2 complexed with its alpha receptor. *Science* 308, 1477–1480
39 Shamri, R. *et al.* (2005) Lymphocyte arrest requires instantaneous induction of an extended LFA-1 conformation mediated by endothelium-bound chemokines. *Nat. Immunol.* 6, 497–506