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30 Years of Biotherapeutics Development—What Have We Learned?

Nico Ghilardi,[1] Rajita Pappu,[1] Joseph R. Arron,[1] and Andrew C. Chan[2]

[1]Department of Immunology, Genentech, South San Francisco, California 94080, USA; email: nico.ghilardi@gmail.com, pappu.rajita@gene.com, arron.joseph@gene.com

[2]Research-Biology, Genentech, South San Francisco, California 94080, USA; email: acc@gene.com

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Abstract

Since the birth of biotechnology, hundreds of biotherapeutics have been developed and approved by the US Food and Drug Administration (FDA) for human use. These novel medicines not only bring significant benefit to patients but also represent precision tools to interrogate human disease biology. Accordingly, much has been learned from the successes and failures of hundreds of high-quality clinical trials. In this review, we discuss general and broadly applicable themes that have emerged from this collective experience. We base our discussion on insights gained from exploring some of the most important target classes, including interleukin-1 (IL-1), tumor necrosis factor α (TNF-α), IL-6, IL-12/23, IL-17, IL-4/13, IL-5, immunoglobulin E (IgE), integrins and B cells. We also describe current challenges and speculate about how emerging technological capabilities may enable the discovery and development of the next generation of biotherapeutics.

INTRODUCTION—GENERAL LESSONS LEARNED

The deciphering of the human genome coupled with advances in biotechnology (1), in particular Köhler and Milstein's (2) landmark discovery of monoclonal antibodies (mAbs), has enabled the generation of precision tools to interrogate biological functions of proteins in health and disease. This collective experience has resulted in over 300 biotherapeutics approved by the US Food and Drug Administration (FDA), of which many are approved for treatment of immunologic and inflammatory diseases (**Table 1**). Much has been learned, and innumerable outstanding review articles have been written about individual biological classes of therapeutics. As we cannot discuss all biological classes of biotherapeutics, nor all of the interesting and important insights derived over the last more than three decades, the focus of this review begins with a reflection on several overarching themes that have emerged from the experience of developing these medicines in the context of autoimmune and inflammatory diseases. These concepts provide important insights into the underlying immune-pathogenic mechanisms for many of these diseases and should guide future drug discovery and development efforts.

Patient and Disease Heterogeneity

Most autoimmune and inflammatory diseases are clinical syndromes defined by a common set of symptoms and diagnostic criteria that arise from a spectrum of molecular causes (**Figure 1*a***). In most diseases, treatment with a single therapy provides clinical benefit in only a subset of patients. Such responders likely share a common disease pathophysiology, whereas nonresponders likely have distinct molecular disease-contributing pathways. Rheumatoid arthritis (RA) and asthma are two examples of diseases with molecular heterogeneity, which is likely a major reason for the failure of any individual therapeutic to achieve efficacy in all patients. Here, diagnostic assessment of disease endotype is necessary to select the most appropriate therapy for each patient subset. In contrast, patients with monogenic diseases [e.g., cryopyrin-associated periodic syndromes (CAPS)] with a single dominant disease effector pathway are more likely to all benefit from a therapeutic that targets a key node in the pathway (e.g., blockade of IL-1β) (**Table 2**). Successful drug development thus requires not only a detailed understanding of the underlying pathophysiology of the human disease but also diagnostic strategies and clinical trial designs that incorporate those insights.

Clinical End Points to Match Biologic Pathways

Heterogeneity may also exist for different clinical manifestations within a patient (**Figure 1*b***). In psoriasis and associated psoriatic arthritis (PsA), targeting the IL-23/17 pathway results in dramatic improvement in cutaneous manifestations. These same agents demonstrate clinical improvement in arthritis, albeit to a lesser degree relative to the benefit observed in the skin, implicating involvement of additional inflammatory pathways affecting the joints. Similarly, IL-5-targeting therapies have a major effect on episodic exacerbations in asthma but do relatively little to improve baseline lung function and symptoms. Hence, different pathophysiological mechanisms can drive distinct clinical manifestations, and for that reason, selection of appropriate clinical end points is of critical importance. This intradisease heterogeneity further suggests that combination therapy may be required to maximize clinical benefit for a given patient in some diseases.

Biomarkers Are Vital for Success in Drug Development

Whether a drug is efficacious depends on its ability to achieve a prespecified clinical end point in a pivotal trial. To improve the likelihood of success, biomarkers are instrumental to maximize clinical benefit and likelihood of success in the drug development process (**Figure 1*c***). Prognostic

Table 1 Biotherapeutics discussed in this review and approved by the FDA for immunologic indications, in pivotal phase 3 clinical trials or positive phase 2 clinical trials listed on clinicaltrials.gov

Target class	Therapeutic	Molecule characteristics	FDA-approved indications for autoimmune or inflammatory indications	Active and recruiting phase 3 or positive phase 2 trials
BAFF/APRIL	Belimumab	Human IgG1λ mAb binding BAFF	SLE	■ Idiopathic inflammatory myositis (P3, NCT 02347891) ■ RA (P2, NCT 00071812)
	Atacicept	TACI-Fc fusion protein binding BAFF and APRIL	Discontinued	–
CD20	Rituximab	Mouse/human chimeric IgG1κ mAb (type I)	RA, GPA, MPA, PV	–
	Ofatumumab	Human IgG1κ mAb with enhanced CDC relative to rituximab (type I)	–	■ RMS (P3, NCT 0350114)
	Ocrelizumab	Humanized IgG1κ mAb with reduced CDC relative to rituximab (type I)	RMS and PPMS	–
	Obinutuzumab	Humanized IgG1κ mAb with enhanced ADCC and apoptosis (type II)	–	■ Lupus nephritis (P2, NCT 02550652)
CD25	Daclizumab	Humanized IgG1κ mAb	Discontinued	–
CD52	Alemtuzumab	Humanized IgG1κ mAb	RMS	–
CD80/CD86	Abatacept	CTLA4-Fc fusion	RA, JIA, PsA	■ GPA (P3, NCT 02108860)
GM-CSF	Otilimab	Human IgG1 mAb	–	■ RA (P3, NCT 03970837, 03980483)
IgE	Omalizumab	Humanized IgG1κ mAb binding free IgE	Allergic asthma, CSU	■ Chronic rhinosinusitis with nasal polyps (P3, NCT 03280537)
	Ligelizumab	Humanized IgG1κ mAb	–	■ CSU (P3, NCT 03580369, 03580356)
IL-1	Anakinra	Aglycosylated human IL-1RA recombinant protein binding IL-1R1	RA, Gout, SAIDs	–
	Canakinumab	Human IgG1κ mAb binding IL-1β	SAIDs	–
	Rilonacept	Dimeric human IL-1R–IL-1RAcP IgG1 fusion protein binding IL-1α and IL-1β	Gout, SAIDs	–
	Ebi-005	Chimeric human IL-1Ra–IL-1β fusion protein binding IL-1R1	–	■ Dry eye disease (P3, NCT 0240539)
	Gevokizumab	Humanized IgG2κ mAb binding IL-1β	–	–
	Bermekimab	Human IgGκ mAb binding IL-1α	–	■ AD (P2, NCT 03496974) ■ HAS (P2, NCT 03512275)
	Ly2189102	Humanized IgG4 mAb binding IL-1β	–	■ RA (P2, NCT 00380744)

(*Continued*)

Table 1 (*Continued*)

Target class	Therapeutic	Molecule characteristics	FDA-approved indications for autoimmune or inflammatory indications	Active and recruiting phase 3 or positive phase 2 trials
IL-5	Mepolizumab	Humanized IgG1κ mAb	Eosinophilic asthma, eosinophilic granulomatosis with polyangiitis	■ Hypereosinophilic syndrome (P3, NCT 02836496)
	Reslizumab	Humanized IgG4κ mAb	Eosinophilic asthma	–
IL-5R	Benralizumab	Humanized afucosylated IgG1 mAb	Eosinophilic asthma	■ Chronic rhinosinusitis with nasal polyps (P3, NCT 03401229)
IL-6/IL-6R	Tocilizumab	Humanized IgG1κ mAb binding IL-6R	RA, GCA, JIA, CRS	■ PMR (P3, NCT 02908217, 03263715) ■ Takayasu arteritis (P3, NCT 02101333)
	Sarilumab	Human IgG1κ mAb binding IL-6R	RA	■ GCA (P3, NCT 03600805) ■ PMR (P3, NCT 03600818)
	Satralizumab	Humanized IgG2 mAb binding IL-6R	–	■ Neuromyelitis optica (P3, NCT 02073279, 02028884)
	Olokizumab	Humanized IgG4 mAb binding IL-6	–	■ RA (P3, NCT 02760433)
	Clazakizumab	Aglycosylated humanized IgG1κ mAb binding IL-6	–	■ Transplant rejection (P3, NCT 03744910)
	Siltuximab	Humanized IgG1κ mAb binding IL-6	Multicentric Castleman disease	–
IL-13 +/− IL-4	Dupilumab	Human IgG4κ mAb binding IL-4Rα to block IL-13 and IL-4 signaling	AD, asthma, chronic rhinosinusitis with nasal polyps	■ Peanut allergy (P2, NCT 03793608, 03682770) ■ Eosinophilic esophagitis (P3, NCT 03633617) ■ COPD (P3, NCT 03930732)
	Lebrikizumab	Humanized IgG4κ mAb binding IL-13	–	■ AD (P2, NCT 02465606, 02340234, 03443024) ■ Asthma (P3, NCT 01867125)
	Tralokinumab	Human IgG4 mAb binding IL-13	–	■ AD (P3 NCT 03363854, 03160885, 03131648, 03526861) ■ Asthma (P3, NCT 02902809)
IL-12/23 (p40)	Ustekinumab	Human IgG1κ mAb	Psoriasis, PsA, CD, UC	■ SLE (P2, NCT 02349061) ■ Graft-versus-host disease (P2, NCT 01713400)

(*Continued*)

Table 1 (*Continued*)

Target class	Therapeutic	Molecule characteristics	FDA-approved indications for autoimmune or inflammatory indications	Active and recruiting phase 3 or positive phase 2 trials
IL-22	Fezakinumab	Human IgG1λ mAb	–	■ AD (P2, NCT 01941537)
IL-23 (p19)	Guselkumab	Human IgG1λ mAb	Psoriasis	■ PsA (P2, NCT 02319759) ■ Palmoplantar pustulosis (P2, NCT 01845987)
	Tildrakizumab	Human IgG1κ mAb	Psoriasis	■ AS (P3, NCT 03552276)
	Risankizumab	Humanized IgG1κ mAb	Psoriasis	■ CD (P3, NCT 03105102, 03105128, 03104413) ■ UC (P3, NCT 03398135, 03398148) ■ PsA (P3, NCT 03675308, 03671148)
	Mirikizumab	Humanized IgG4κ mAb	–	■ UC (P3, NCT 03519945, 03518086, 03524092) ■ Psoriasis (P2, NCT 02899988)
	Brazikumab	Human IgG2λ mAb	–	■ CD (P2/3, NCT 03759288, 01714726)
IL-17A	Secukinumab	Human IgG1κ mAb	Psoriasis, PsA, AS	■ HAS (P3, NCT 03713632, 03713619) ■ RA (P3, NCT 01377012, 01350804)
	Ixekizumab	Humanized IgG4κ mAb	Psoriasis, PsA, AS	■ RA (P2, NCT 00966875)
IL-17A/F	Bimekizumab	Humanized IgG1κ mAb	–	■ Psoriasis (P3, NCT 03410992, 03370133) ■ PsA (P3, NCT 03896581, 03895203)
IL-17Ra	Brodalumab	Human IgG2κ mAb	Psoriasis	■ PsA (P3, NCT 02024646) ■ Spondyloarthritis (P3, NCT 02985983) ■ Systemic sclerosis (P3, NCT 03957681)

(*Continued*)

Table 1 (*Continued*)

Target class	Therapeutic	Molecule characteristics	FDA-approved indications for autoimmune or inflammatory indications	Active and recruiting phase 3 or positive phase 2 trials
Integrins	Natalizumab	Humanized IgG4 mAb blocking $\alpha_4\beta_1$ and $\alpha_4\beta_7$	RMS, UC	–
	Vedolizumab	Humanized IgG1 mAb blocking $\alpha_4\beta_7$ interactions with MADCAM-1 and VCAM	UC, CD	■ Graft-versus-host disease (P3, NCT 03657160)
	Etrolizumab	Humanized IgG1 mAb blocking $\alpha_E\beta_7$ and $\alpha_4\beta_7$	–	■ UC (P3, NCT 02165215, 02118584, 02171429, 02163759, 02136069, 02100696) ■ CD (P3, NCT 02394028, 02403323)
	Abrilumab	Human IgG2κ mAb targeting $\alpha_4\beta_7$	–	■ CD (P2, NCT 01696396) ■ UC (P2, NCT 01694485)
	PF-00547659	Human IgG2 mAb blocking MAdCAM1	–	■ CD (P3, NCT 03566823, 03559517)
TNF	Infliximab	Chimeric mouse/human IgG1κ mAb	CD, UC, RA, AS, psoriasis, PsA	–
	Etanercept	TNFRII-Fc fusion protein inhibiting both TNF-α and TNF-β	RA, JIA, psoriasis, AS	–
	Adalimumab	Human IgG1κ mAb	RA, JIA, psoriasis, PsA, AS, CD, UC, hidradenitis suppurativa, uveitis	–
	Golimumab	Human IgG1κ mAb	RA, PsA, UC	■ Axial spondyloarthropathy (P3, NCT 03270501)
	Certolizumab pegol	Pegylated Fab	UC, RA, PsA, AS	–
TSLP	Tezepelumab	Human IgG2λ mAb	–	■ Asthma (P3, NCT 03347279) ■ AD (P2, NCT 02525094, 03809663)

– indicates none known.

Abbreviations: AD, atopic dermatitis; AS, ankylosing spondylitis; ADCC, antibody-dependent cell-mediated cytotoxicity; CD, Crohn disease; CDC, complement-dependent cytotoxicity; COPD, chronic obstructive pulmonary disease; CRS, cytokine release syndrome; CSU, chronic spontaneous urticaria; FDA, US Food and Drug Administration; GCA, giant cell arteritis; GPA, granulomatosis with polyangiitis; HAS, hidradenitis suppurativa; JIA, juvenile idiopathic arthritis; mAb, monoclonal antibody; MPA, microscopic polyangiitis; P2, phase 2; P3, phase 3; PMR, polymyalgia rheumatica; PPMS, primary progressive multiple sclerosis; PsA, psoriatic arthritis; PV, pemphigus vulgaris; RA, rheumatoid arthritis; RMS, relapsing multiple sclerosis; SAID, systemic autoinflammatory disease; SLE, systemic lupus erythematosus; TNF, tumor necrosis factor; TSLP, thymic stromal lymphopoietin; UC, ulcerative colitis.

Table 2 Clinical efficacy by disease and target class

Disease	BAFF/APRIL	CD20	CD80/86	TNF	IL-1	IL-6	IgE	IL-5/IL-5R	IL-4/13	IL-12/23 (p40)	IL-23 (p19)	IL17A/F and IL-17R	α_4 integrin	$\alpha_4\beta_7/\alpha_E\beta_7$ integrins
Atopic dermatitis									++					
Asthma				−			+	+	++			−		
COPD				−				−	−					
Multiple sclerosis	−	++		−						−		+	++	
Psoriasis				+						+	++	++		
Psoriatic arthritis				+						+	+	+		
Crohn's disease				+						++	+	−	+	+
Ulcerative colitis				+					−	+				+
Ankylosing spondylitis				+						−	−	+		
RA		+	+	+	+	+				−	−			
SLE	+	+				−								
SAIDs					++									

− indicates no clinical effect or worsened disease; + indicates clinical benefit observed in pivotal or randomized phase 2 clinical studies; ++ indicates profound clinical benefit (e.g., ACR70 for RA) achieved for >50% of patients. Empty cells indicate the lack of randomized placebo-controlled phase 2 or 3 clinical studies for the target class in the disease.

Abbreviations: COPD, chronic obstructive pulmonary disease; RA, rheumatoid arthritis; SAID, systemic autoinflammatory disease; SLE, systemic lupus erythematosus; TNF, tumor necrosis factor.

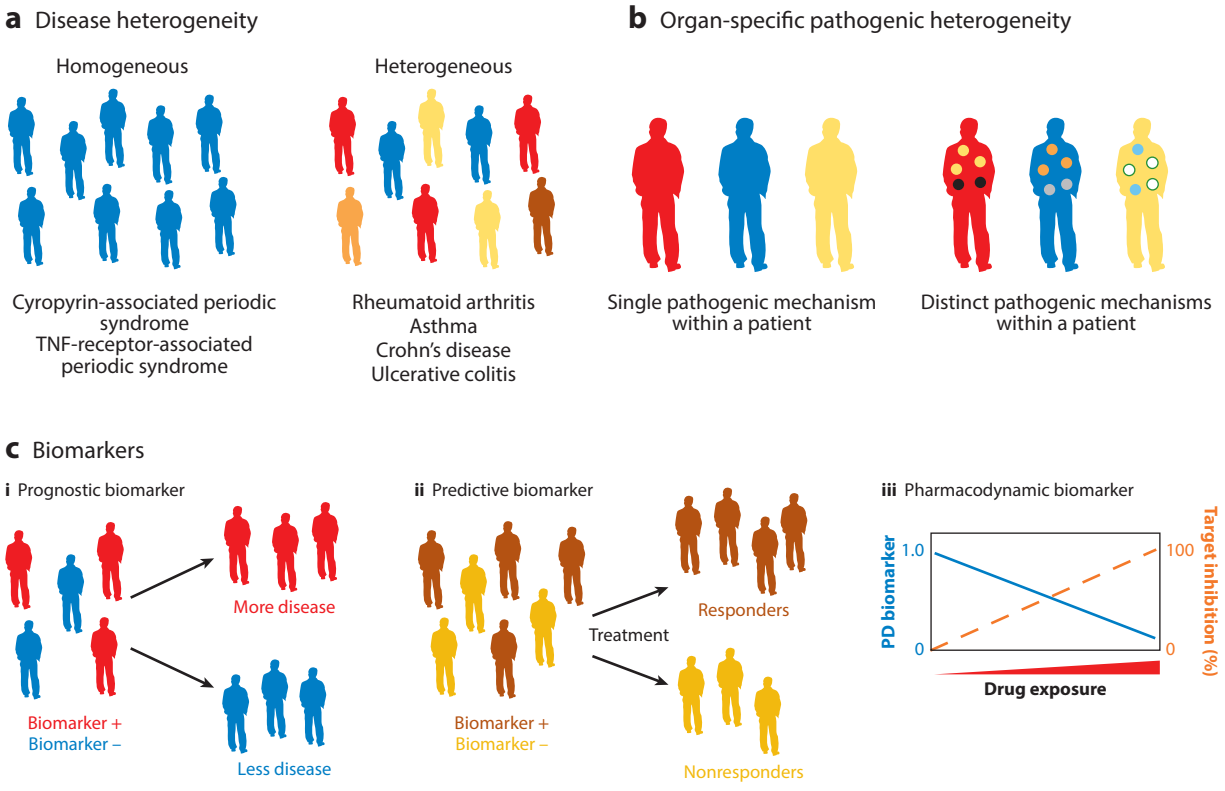

Figure 1

General themes emerging from clinical interventional trials of immune and inflammatory diseases. (*a*) Disease heterogeneity. Homogeneous diseases have a common pathogenic mechanism. Monogenic diseases, such as CAPS or TRAPs, have a common effector pathway and hence have profound clinical responses to IL-1 antagonists (**Table 2**). Heterogeneous diseases have multiple pathogenic mechanisms. In turn, patients, in aggregate, do not uniformly respond to a single therapy. (*b*) Organ-specific pathogenic heterogeneity. Within each patient, a single disease mechanism may give rise to all clinical manifestations (*left*), or different disease mechanisms may be responsible for different clinical manifestations within a given patient (*right*). (*c*) Biomarkers: (*i*) Prognostic markers can identify a patient subset at baseline that will have worse clinical manifestations over time than biomarker-negative patients. (*ii*) Predictive biomarkers can identify, at baseline, patients who have a preferential response to a given therapy. (*iii*) Pharmacodynamic markers measure the degree of inhibition of the target at a specific dose. Abbreviations: CAPS, cryopyrin-associated periodic syndromes; PD, pharmacodynamic; TRAP, TNF-receptor-associated periodic syndrome.

biomarkers identify patient subgroups that have a different clinical course than others with the same disease. Predictive biomarkers can be used to identify patients, prior to therapy, who are more likely to benefit from a therapeutic. Pharmacodynamic biomarkers report the ability of a drug to engage its target, providing vital information about appropriate drug dose and schedule. Use of predictive, prognostic, and pharmacodynamic biomarkers in drug development can mitigate the issues of patient heterogeneity, enrich for clinical outcome measures, and determine whether sufficient target inhibition has been achieved, respectively.

Limitations of Genetic Deficiencies as Predictors of Clinical Efficacy or Safety

Single-nucleotide polymorphisms (SNPs) as well as naturally occurring or artificially generated genetic alterations can lead to gain- or loss-of-function (partial or complete) alleles. Analysis of the resulting phenotypes provides important insights into the function of genes. However, there

are limitations in using these insights to guide disease indications or safety concerns for drug development. These include

1. Differences exist in disease pathogenesis during disease initiation and effector phases. Since most clinical trials involve symptomatic patients, therapies that interfere with pathways involved in disease initiation are unlikely to be effective during the effector phase, if their pathogenic mechanisms differ.
2. In assessing potential safety concerns, gene-deficient organisms (humans included) can exhibit marked sensitivities to infectious challenges. However, patients who present with a disease during adulthood have the benefit of existing immunity as a consequence of environmental and infectious exposures or immunizations incurred prior to therapeutic intervention. Accordingly, therapeutics inhibiting tumor necrosis factor-α (TNF-α), IL-6, or IL-12/23 are much safer than genetically compromised humans or mice would predict.
3. Animals housed under specific-pathogen-free (SPF) conditions, typically used for preclinical investigation, have immune systems that resemble that of a newborn human more than that of an antigen-experienced adult patient and may falsely predict the biological consequences of therapeutic interventions to the adult human organism in real-world settings (3). For example, the microbiota of SPF-housed mice differs from that of mice living in natural environments, and mice harboring a natural microbiota recapitulate better than mice housed under SPF conditions the clinical effects observed with CD28-superagonist and anti-TNF-α mAbs in human clinical trials (4).
4. Finally, while complete inactivation of a gene may be harmful, small-molecule inhibitors with substantially shorter half-lives can be used at doses that do not achieve full target inhibition to maximize therapeutic index (i.e., the ratio of dose providing efficacy to toxicity), as exemplified by Janus kinase (Jak) inhibition (5).

Lessons from Negative Clinical Trials

Positive clinical trial outcomes, particularly when reproduced with different agents within the same therapeutic class, allow for greater confidence to support a key biologic role for a pathway in disease. For example, the preponderance of data supports important roles of TNF-α in a number of inflammatory disorders. However, negative clinical trial outcomes do not necessarily mean that a biological pathway is not related to the disease. At least four major reasons can contribute to clinical trial failure:

1. Drug design. Inadequate molecular properties of the drug candidate, including target specificity, candidate affinity and potency, off-target effects that give rise to adverse events that limit on-target inhibition, poor drug biodistribution to disease sites of action (e.g., brain), and poor pharmacokinetic properties, may lead to a negative outcome despite the mechanistic contribution of the target to disease pathogenesis. Availability and stringent application of sensitive pharmacodynamic biomarkers can typically identify these causes.
2. Study design. Failure to appropriately set entry criteria for a study population with sufficient disease activity or likelihood of disease progression within the study duration may negatively impact the sensitivity of a clinical trial to detect a treatment effect. Failure to account for clinical efficacy in the placebo group, who will receive concomitant standard-of-care (SOC) medications and maintain a higher level of compliance, will also reduce the therapeutic window to measure efficacy. Finally, matching appropriate clinical end points to the targeted biological pathway is critical in multifactorial disorders with heterogeneous clinical presentations.

3. Patient selection. Disease heterogeneity will dilute therapeutic benefit in trials enrolling all-comer populations. In phase 2 proof-of-concept trials, where a diagnostic hypothesis is typically tested, patient numbers need to be increased (to incorporate both diagnostic-positive and -negative patients) in order to provide appropriate power to detect clinical benefit in the diagnostic-positive patient subset, validate the diagnostic hypothesis, and, in many cases, identify a suitable cutoff for defining diagnostic positivity (**Figure 1*a*,*c***).
4. Target selection. A well-designed trial should allow for ruling out reasons 1–3, and if that is possible, a negative result likely means that despite preclinical information that led to the selection of the target for the given indication, the therapeutic hypothesis was indeed wrong.

THERAPEUTIC CLASSES

In this section, we expand on these general themes through specific examples from several major target classes. We begin each with a synopsis of the underlying biology, discuss therapeutics available within the class, and thereafter describe specific immunologic or clinical insights derived from interventional studies in the clinic.

Interleukin-1

IL-1α and IL-1β are positioned to respond rapidly to both sterile and microbially mediated inflammation. IL-1α acts as an alarmin when released from epithelial cells, endothelial cells, and platelets in response to tissue damage (6, 7). In contrast, the myeloid-derived isoform, IL-1β, is synthesized as an inactive protein that requires inflammasome-driven proteolytic processing for activity. IL-1α/β belong to the larger IL-1 cytokine family, which use the common IL-1 receptor accessory protein (IL-1RAcP) paired with the IL-1R1 subunit to elicit MyD88-dependent signals. IL-1α/β functions are also regulated by the natural antagonists IL-1Ra, sIL-1RAcp, and decoy receptor IL-1R2.

Both pathogen-associated molecular patterns (PAMPs) [e.g., lipopolysaccharide (LPS), muramyl dipeptide, viral nucleic acids, and flagellin] and damage-associated molecular patterns (DAMPs) (e.g., ATP, uric acid, calcium pyrophosphate, and cholesterol crystals) activate the inflammasome, resulting in cell death and the release of bioactive IL-1β (8). IL-1α/β are potent pyrogens that stimulate chemokine production to recruit neutrophils and monocytes; they also promote T helper type 17 (Th17) differentiation. IL-1α/β induce proinflammatory effects on vascular endothelial and smooth muscle cells, including production of IL-6, upregulation of adhesion molecules, and induction of chemokines/cytokines to contribute to atherogenesis. Finally, IL-1α/β play important roles in bone health by inducing osteoclast differentiation.

Dysregulated IL-1α/β expression is associated with a multitude of systemic autoinflammatory diseases (SAIDs) characterized by fever, rash, arthritis, and organ-specific inflammation. Mutations in *NLRP3* (CAPS), *IL1RN* (deficiency of IL-1 receptor antagonist), and other genes can lead to uncontrolled IL-1β activity (9, 10). Accordingly, IL-1β antagonists have demonstrated excellent clinical efficacy for SAIDs and are an example of one therapeutic class that provides benefit to nearly all patients of a homogeneous syndrome with a common disease effector pathway.

IL-1-targeting therapeutics. Three biotherapeutics with distinct properties, but all targeting IL-1, have been approved by the FDA (**Table 1**). Anakinra, a modified version of the natural antagonist IL-1Ra, inhibits both IL-1α and IL-1β (10). Anakinra binds to IL-1R1 and prevents the recruitment of IL-1AcP to produce a nonproductive signaling complex at the plasma membrane.

Anakinra, approved for treatment of RA and CAPS, has a short half-life of 5–6 h and therefore requires daily subcutaneous (sc) injections (11, 12). Anakinra is also effective in gout, where monosodium urate crystals stimulate NLRP3 activation and IL-1β production. Canakinumab, an IL-1β-neutralizing IgG1κ mAb administered sc every 4 to 8 weeks, is approved for treatment of systemic juvenile idiopathic arthritis (SJIA) and SAIDs (13, 14). Rilonacept, encoding the extracellular domains of IL-1R and IL-1AcP linked to the Fc portion of IgG, preferentially neutralizes IL-1β and is approved for weekly sc administration for SAIDs (15).

IL-1β in atherosclerosis, cancer, and osteoarthritis. Because chronic inflammation is highly associated with risk of cardiovascular events independent of cholesterol level, the Canakinumab Anti-inflammatory Thrombosis Outcome Study (CANTOS) was designed to test whether IL-1β inhibition would prevent recurrent vascular events (i.e., nonfatal myocardial infarction, nonfatal stroke or cardiovascular death) (16). Canakinumab demonstrated dose-dependent decreases in high-sensitivity C-reactive protein and serum IL-6 levels, without any effect on low-density lipoprotein (LDL) or high-density lipoprotein (HDL) cholesterol levels. At a median follow-up of 3.7 years, patients treated with 150 or 300 mg every three months demonstrated significant reductions in major adverse cardiovascular events (MACEs). Highlighting the importance of pharmacodynamic biomarkers, patients who achieved on-treatment IL-6 levels below the study median value of 1.65 ng/L experienced a 32% reduction in MACEs, whereas those with on-treatment IL-6 levels $\geq$1.65 ng/L derived no significant benefit. It remains to be determined whether higher or more frequent doses of canakinumab might have been more efficacious in the latter group (17).

Consistent with the importance of IL-1β in host defense, canakinumab treatment resulted in a small but statistically significant increase in mortality due to infection or sepsis when compared to placebo (16). This increase was unexpectedly balanced by a significant reduction in total cancer mortality. Incidence of lung, but not nonlung, cancer was significantly reduced (18). The precise mechanism by which neutralization of IL-1β protected against lung cancer is not known, but the finding is consistent with the hypothesis that inflammation can promote tumor formation and prompts a new line of investigation of IL-1β in cancer (19, 20). In addition to its effect on lung cancer development, canakinumab-treated patients also experienced lower incidences of gout and osteoarthritis (16, 21). Thus, due to its size and duration, the CANTOS study enabled the serendipitous discovery of important in vivo functions of IL-1β in humans, which were not primary end points of the trial. More will be learned from IL-1 targeting in ongoing and future clinical trials for additional diseases.

Interleukin-6

IL-6, a member of the gp130 cytokine family, is a central component of many homeostatic and inflammatory processes (22, 23). Originally identified as a T cell–derived factor that promotes B cell differentiation, IL-6 is now appreciated for its pleiotropic activities within and beyond adaptive immunity. It contributes to the differentiation of Th17 and T follicular helper (Tfh) cells, drives myeloid cell differentiation, and synergizes with Th2 cytokines to promote macrophage polarization to a profibrotic phenotype. IL-6 plays a central role in the hepatic acute phase response, increasing inflammatory proteins including C-reactive protein (CRP) to enhance complement binding to pathogens or dying cells, as well as important effects on endothelial cell function and epithelial cell integrity.

The broad biological activities of IL-6 arise from the complex nature of its regulation and its multiple cellular targets (24). IL-6 is synthesized by a multitude of cells and activates target cells through three distinct cell surface signaling mechanisms (i.e., classical signaling, *trans*-signaling and *trans*-presentation) culminating in JAK/STAT signaling.

Tocilizumab and sarilumab are two FDA-approved IL-6R-blocking mAbs, and many additional agents are in clinical development (**Table 1**). Tocilizumab first demonstrated efficacy in multicentric Castleman disease, a lymphoproliferative disease with benign hyperplastic lymph nodes. Symptoms of fever and fatigue and abnormalities in CRP, albumin, and immunoglobulin levels all abated with tocilizumab therapy. Tocilizumab also provides substantial clinical benefit in SJIA and giant cell arteritis, a chronic granulomatous vasculitis involving both large and medium blood vessels with a multicellular inflammatory infiltrate (25, 26). Tocilizumab and sarilumab are approved by the FDA for treatment of moderate to severe RA, where IL-6 levels are elevated in synovial fluid of affected joints, and where serum IL-6 levels correlate with disease activity. Both agents improve inflammatory symptoms and decrease radiographic progression in patients with RA; the latter may be related to blockade of IL-6-mediated RANKL induction, which drives osteoclastogenesis and bone erosion (27).

Cytokine release syndrome (CRS) is an acute systemic inflammatory condition associated with many antibody-based therapies, chemotherapy, and T cell–engaging immunotherapies (e.g., chimeric antigen receptor–modified T cells) as well as occurring following severe infection (28). CRS associated with T cell–engaging therapies is thought to result from production of TNF-α by activated T cells, which in turn triggers IL-6 and IL-1β production by monocytes and activated macrophages (29). Serum IL-6, sIL-6R, interferon-γ (IFN-γ), and soluble gp130 levels correlate with CRS severity, which ranges from mild to lethal. A hallmark of severe CRS appears to be mediated through *trans*-presentation of IL-6 to endothelial cells, as they express gp130 but not mIL-6R. Resolution of CRS symptoms (e.g., fever and hypotension) is often achieved following a single dose of tocilizumab (30). Administration of tocilizumab does not appear to affect the antitumor effect of T cell–engaging therapies.

The faSScinate study evaluated tocilizumab in systemic sclerosis (SSc), a connective tissue disease affecting skin, lung, gastrointestinal tract, blood vessels, and kidney, with elements of autoimmunity, vasculopathy, and tissue fibrosis (31). While the primary end point—improvement in the Modified Rodnan Skin Score at 24 weeks—was not achieved, tocilizumab reduced the rate of lung function decline as measured by forced vital capacity at 48 weeks. As a majority of patients with diffuse cutaneous SSc develop interstitial lung disease, this benefit of IL-6 inhibition is potentially clinically meaningful. Mechanistic clues to tocilizumab's effect on SSc disease progression arise from pharmacodynamic biomarker effects: Tocilizumab treatment decreased serum levels of the macrophage-derived chemokine CCL18 and skin M2-macrophage-associated genes when compared to placebo, whereas it did not affect skin TGF-β or IFN-α gene clusters or serum fibrosis biomarkers—ENPP2, COMP, or POSTN (32). Although IL-6 activates many pathways relevant to SSc, the effect on CCL18 suggests that IL-6R blockade impacts the ability of pulmonary macrophages to contribute to interstitial lung disease progression, while only modestly impacting fibroblast biology. This selective improvement on lung function is an example of IL-6-mediated organ-specific disease pathogenesis within a multiple-organ disease.

Although IL-6 is a potent, pleiotropic mediator of inflammation, it also plays roles in tissue homeostasis. In the intestinal epithelium, IL-6 is produced by lamina propria myeloid cells and intestinal epithelial T lymphocytes (IELs). It provides antiapoptotic signals and stimulates epithelial proliferation following injury and is thus critical for epithelial repair (33). Cautious use of tocilizumab and sarilumab is advised in patients with intestinal inflammation and those with concomitant use of nonsteroidal anti-inflammatory drugs or corticosteroids, as gastrointestinal perforations were observed in early clinical trials with both agents (34, 35). More will be learned about the pleiotropic functions within and beyond adaptive immunity from ongoing and future clinical trials with IL-6 antagonists.

Tumor Necrosis Factor

Observations that cancer patients who develop bacterial infections sometimes experienced spontaneous tumor regressions date back to the 1700s and prompted William Coley in the 1800s to treat cancer by injecting *Streptococcus* or a combination of heat-killed *Streptococcus* and *Serratia marcescens* (Coley's toxin) to mimic bacterial infections (36). TNF was coined for a serum activity that killed implanted sarcoma 37 tumors in mice treated with *S. marcescens* polysaccharide (37). Similarly, hemorrhagic necrosis of tumors due to TNF activity can be induced in Bacillus Calmette-Guérin–infected mice treated with endotoxin (38). These observations and subsequent molecular cloning ultimately resulted in the identification of TNF-α (also known as cachexin) and lymphotoxin-α (LTα) (later termed TNF-β) (39–41).

TNF-α is produced predominantly by immune and endothelial cells and is significantly upregulated with proinflammatory signals and bacterial products; TNF-β/LTα is produced primarily by lymphocytes (42). TNF-α is expressed as a trimeric membrane-bound form (mTNF) and undergoes proteolytic cleavage to give rise to the soluble trimeric sTNF. TNFR1 is ubiquitously expressed, while TNFR2 is predominantly expressed on neurons, immune cells, and endothelial cells.

TNF-α has pleiotropic functions (42). TNF-α is required for optimal defense against pathogens and proper lymphoid organ development as well as important reparative roles in neuronal remyelination, cardiac remodeling, and cartilage regeneration. *Tnfα*$^{-/-}$ mice lack primary splenic B cell follicles and have highly disorganized follicular dendritic cell networks and germinal centers (GCs). *Tnfβ*$^{-/-}$ mice also have disorganized splenic architecture and defective secondary lymphoid organ development. *Tnfr1*$^{-/-}$ mice are highly resistant to low-level LPS challenge and have increased susceptibility to *Listeria* infection. *Tnfr2*$^{-/-}$ mice demonstrate increased sensitivity to bacterial pathogens, decreased sensitivity to LPS, and decreased antigen-induced T cell apoptosis.

TNF inhibitors. Five TNF inhibitors are presently FDA approved for human use (**Table 1**). All target TNF-α, and etanercept additionally inhibits TNF-β. The molecular types of TNF antagonists reflect the evolution of biotherapeutic drug development over the last two decades. Etanercept (TNFR2-Fc) was the first Fc-fusion protein approved by the FDA; infliximab is a first-generation chimeric mAb; adalimumab is a human mAb derived from phage display; golimumab is a human mAb generated from genetically modified mice expressing human IgG; certolizumab pegol is a humanized Fab fragment isolated from a mouse hybridoma and pegylated to extend its in vivo half-life. It is noteworthy that although TNF inhibition is associated with increased risk of infections, particularly by *Mycobacterium tuberculosis*, the adverse effect profile observed clinically is far less serious than what would be predicted from *tnf*$^{-/-}$ mice (with proper patient screening for *M. tuberculosis*).

Most TNF antagonists demonstrate a shared spectrum of clinical efficacy. However, etanercept was ineffective in a randomized double-blind, placebo-controlled trial for Crohn's disease (CD) and may reflect differences in the therapeutic mechanisms of TNF inhibitors (43, 44). In addition to neutralizing sTNF-α, anti-TNF antibodies and certolizumab pegol, but not etanercept, can induce lamina propria T cell apoptosis by binding mTNF or interrupting antiapoptotic signals. Furthermore, anti-TNF mAbs, but not etanercept or certolizumab pegol, can induce M2-type wound healing macrophage responses through an Fc-dependent mechanism. Hence, mechanistic differences between TNF antagonists may account for their divergent clinical effects in CD.

Protective functions of TNF. While TNF antagonists demonstrate clinical benefit in numerous autoimmune and inflammatory conditions, treatment with TNF antagonists worsens relapsing

multiple sclerosis (RMS). In a randomized double-blind, placebo-controlled study of 168 patients with RMS with lenercept, a TNFR1-Fc fusion protein, patients demonstrated a dose-dependent increase in clinical relapse and severity of exacerbations (45). In addition, anti-TNF therapies are also associated with increased risks of optic neuritis and other central and peripheral demyelinating diseases (46).

This paradox of TNF inhibitors across diseases is due to their anti-inflammatory effects balanced by their protective functions in tissue homeostasis. Studies in transgenic or knockout mice indicate that sTNF-α through TNFR1 promotes inflammation and increases blood-brain barrier permeability and demyelination. Conversely, TNF-α has been demonstrated to inhibit development of peripheral encephalitogenic Th1 and Th17 cells (47). More importantly, mTNF/TNFRII interactions play an important role in remyelination as well as proliferation and differentiation of oligodendrocyte progenitor cells into mature oligodendrocytes (48). Hence, in RMS, the protective remyelination role of TNF-α appears to outweigh the anti-inflammatory benefits of TNF-α inhibition.

Treatment with anti-TNF therapy is also associated with development of antinuclear and anti-dsDNA autoantibodies as well as rare development of systemic lupus erythematosus (SLE)-like syndromes and vasculitis (49, 50). There is an intricate interplay between TNF-α and interferons. TNF-α inhibits maturation of plasmacytoid dendritic cells (pDCs), a major source of IFN-α/β, from $CD34^+$ hematopoietic stem cells (HSCs), and also inhibits IFN-α induction in virally infected, immature pDCs (51, 52). In turn, patients treated with TNF antagonists demonstrate increased expression of IFN-α/β-regulated genes and increased risk for SLE-like syndromes and paradoxical psoriasis. Despite elevated levels of TNF-α in the airways of asthma patients and signs of benefit from etanercept in a small open-label study (53), TNF inhibition with golimumab failed to demonstrate significant clinical benefit in a phase 2 randomized controlled trial for severe asthma, and an increased rate of serious infections in the golimumab-treated patients led to a premature stop to the trial (54). Hence, understanding the homeostatic functions of targets is important in balancing therapeutic benefit and potential adverse toxicities. Whether selective targeting of TNFRI or developing end-organ-specific inhibition of TNF can provide benefit while limiting toxicities requires additional investigation.

B Cells

B cells can contribute to autoimmune and inflammatory diseases in several ways: They produce antibodies, which can mediate pathology directly by binding to self-antigens and indirectly by forming immune complexes; they present internalized processed antigens via surface MHC class II molecules, along with costimulatory cell surface ligands, to T cells, thereby activating T cell responses; and they can produce proinflammatory cytokines and chemokines, which may further amplify innate and adaptive immune responses.

Anti-CD20 monoclonal antibodies. CD20 is a B-lineage cell marker with expression beginning on early pre-B cells, but lost during terminal differentiation to plasma cells. It is a tetraspanin protein and is required for optimal B cell function and immune responses (55). Two types of anti-CD20 mAbs have been defined based on their ability to redistribute CD20 into detergent-resistant microdomains, also termed lipid rafts. Type I mAbs induce CD20 to redistribute to rafts, while type II anti-CD20 mAbs do not. Clustering of type I mAbs to lipid rafts increases antibody avidity resulting in improved complement-dependent cytotoxicity (CDC). Type II mAbs are relatively ineffective in CDC, but they can directly induce programmed cell death (56). This nonapoptotic form of cell death is induced through homotypic adhesion, actin redistribution, lysosomal swelling,

and dispersal of lysosomal contents resulting in loss of plasma membrane integrity. Type I and type II mAbs also demonstrate different CD20 binding stoichiometries and configurations. Both type I and type II mAbs are capable of antibody-dependent cell phagocytosis and antibody-dependent cell-mediated cytotoxicity (ADCC).

Rituximab, a chimeric mAb, was the first anti-CD20 mAb approved by the FDA for treatment of B cell non-Hodgkin lymphoma. Based on its surprisingly favorable safety profile in oncology, investigators began to explore its use in patients with severe autoimmune disorders. Rituximab is presently approved by the FDA for treatment of a number of autoimmune diseases (**Table 1**). Much insight into human immunology has been gained from rituximab and other anti-CD20 mAbs. Since CD20 is expressed on neither HSCs nor terminally differentiated plasma cells, selective targeting of $CD20^+$ B cells by anti-CD20 mAbs has different immunologic consequences than congenital B cell deficiencies. For example, serum IgG levels are not significantly affected following treatment with anti-CD20 antibodies (see below), whereas an absence of immunoglobulins is observed with severe X-linked agammaglobulinemia, in which B cells do not develop.

Depletion by type I anti-CD20 mAbs is primarily mediated through FcR-mediated clearance by the reticuloendothelial system (57). Studies in both mice and humans have demonstrated that not all B cells are depleted. The B cell microenvironment significantly contributes to the sensitivity of B cell depletion. Factors, including BAFF/BLys or integrins, can provide survival signals to alter depletion sensitivity. In RA patients treated with rituximab, peripheral B cells are consistently and profoundly depleted, but depletion of synovial and bone marrow B cells is highly variable and typically only modestly affected (58–60). Treatment of SLE patients with rituximab exhibits even greater variability in B cell depletion as compared to treatment of RA patients, with a substantial percentage of patients not achieving depletion to <1% of total peripheral blood lymphocytes or still having >5 B cells/μL (61, 62). In preclinical models, splenic B cells also demonstrate significantly greater resistance to depletion in autoimmune-prone compared to non-autoimmune-prone mouse strains (63). SLE patients with less complete B cell depletion or faster repletion have less clinical benefit from rituximab (64). While rituximab or ocrelizumab did not provide additional clinical benefit compared to SOC in randomized placebo-controlled phase 3 clinical trials (65), a recent phase 2 study with obinutuzumab, a type II anti-CD20 mAb with enhanced ADCC and ability to induce B cell apoptosis, has reported clinical benefit in patients with lupus nephritis (NCT 02550652). Additional investigation with obinutuzumab will be required to confirm whether additional B cell depletion will provide benefit in lupus nephritis.

Effects on serum antibodies and autoantibodies. As mature plasma cells do not express CD20, clinical studies with anti-CD20 antibodies have provided important insights into antibody-secreting cells. In phase 3 clinical trials of rituximab in RA (66), median IgG and IgA levels remain stable, and <1% of patients develop IgG and IgA levels below the lower limit of normal (LLN) following therapy. While median IgM levels also remain stable and within the normal range, ~5% of RA patients versus 1.9% of control patients had IgM levels below LLN after treatment. In long-term follow-up studies of 2,578 patients with RA treated with rituximab in combination with methotrexate based on 5,013 patient-years of rituximab exposure (without a control arm), the proportion of patients with IgM < LLN increased with the number of treatment courses, while the proportion of patients with IgG < LLN remained stable over time and independent of number of treatment courses (67). The differential effects on IgM versus IgG/IgA may reflect differences in half-life and the microenvironments of IgM versus IgG/IgA plasma cells. Immunoglobulin levels below LLN were not associated with serious infections. Older age was the only independent predictor of serious infections and sustained low IgG.

In contrast to RA patients, rituximab-treated patients with granulomatosis with polyangiitis (GPA) (formerly known as Wegener's granulomatosis) and microscopic polyangiitis (MPA), which are forms of antineutrophil cytoplasmic antibody vasculitis (AAV), experience delayed B cell repopulation kinetics and higher rates of hypogammaglobulinemia (68). In a retrospective analysis of 239 patients with AAV treated continuously with rituximab (median of 2.4 years, range 1.5 to 4 years), serum IgG levels of rituximab-treated AAV patients decreased at a mean rate of 6% per month. New significant hypogammaglobulinemia (IgG < 400 mg/dL) occurred in 4.6% of patients, all of whom began with the lowest baseline serum IgG quartile, and was independently associated with serious infections. These data indicate differences in plasma cell homeostasis between diseases and require additional mechanistic investigation.

In studies of RA, preexisting antibody titers to tetanus, diphtheria, measles, mumps, and rubella are unaffected by rituximab (69). Recall responses to tetanus or diphtheria are reduced when compared to recall responses in untreated patients, though rituximab-treated patients retain the ability to induce a protective response. In contrast, antibody response to neoantigens (e.g., phiX174) in rituximab-treated patients is inhibited, though immune responses return toward normal following B cell repletion. In contrast to preexisting antibodies to foreign antigens, autoantibodies are highly varied in response to B cell depletion. Highly sensitive autoantibodies (e.g., antidesmoglein 1 and 3 autoantibodies in pemphigus vulgaris or antiphospholipase A2 receptor autoantibodies in membranous glomerulonephritis) likely arise from $CD20^+$ plasmablasts or short-lived $CD20^-$ plasma cells requiring repopulation from autoreactive $CD20^+$ B cells. In the K/BxN mouse model, anti-GPI-producing plasmablasts express low levels of CD20 (70). In addition, TLR9 activation of $CD20^+CD27^+$ memory B cells can sustain CD20 expression as they differentiate to pre-plasmablasts/plasmablasts (71). IgG4 isotype autoantibodies are also highly sensitive to rituximab treatment (72). In contrast, autoantibodies or antibodies arising from long-lived $CD20^-$ plasma cells (e.g., antinuclear or antiextractable nuclear antigen autoantibodies in SLE) are more resistant to anti-CD20 treatment. These long-lived plasma cells do not appear to be dependent on repopulation by $CD20^+$ B cells, but they can be sustained by other activators, including Toll-like receptors (TLRs) and APRIL/BAFF plasma cell survival factors (73).

BAFF/APRIL Tumor Necrosis Factor Superfamily Members

BAFF and APRIL, two type II transmembrane proteins that are members of the TNF ligand superfamily, and their receptors, BAFFR, BCMA, and TACI, play important roles in the survival and maturation of B-lineage cells and functions (74). BAFF and APRIL are produced as homotrimers, though BAFF/APRIL heterotrimers have also been described. While APRIL is primarily produced as a soluble form, BAFF is first expressed as a membrane-bound form (mBAFF) that undergoes furin cleavage, giving rise to soluble BAFF (sBAFF). BAFF and APRIL both bind to BCMA and APRIL, while BAFF also binds to a private BAFFR. *Baff*$^{-/-}$ mice lack mature B cells beyond the transitional stage 1 and have impaired humoral responses. Mice expressing a furin-resistant form of BAFF demonstrate similar defects. mBAFF appears to independently regulate and contribute signals for development of peritoneal B2 and marginal zone B cells. *April*$^{-/-}$ mice demonstrate normal T and B cell development but have defective IgA class switching. Conversely, BAFFR is required for survival and maturation of B cells, BCMA for plasmablast and plasma cell survival, and TACI for T-independent immune responses and class switching.

Serum BAFF and APRIL levels are elevated in a number of autoimmune disorders including SLE, Sjögren syndrome, and RA. Belimumab is a human IgG1λ mAb approved for treatment of active autoantibody-positive SLE (75). It inhibits sBAFF trimers but does not bind to mBAFF or APRIL. In pivotal clinical trials, belimumab demonstrated greater improvement in the SLE

responder index and health-related quality-of-life end points when compared to the SOC group. In addition, belimumab decreased the number of circulating naive B cells, activated B cells, and plasma cells; anti-dsDNA levels were reduced and complement levels were normalized. Consistent with the lack of effect on circulating memory B and T cells, preexisting antipneumococcal, tetanus, and influenza A antibodies were not affected after one year of therapy.

The overlapping functions of BAFF and APRIL on long-lived plasma cell survival is underscored by the lack of effects of either BAFF or APRIL deficiency on homeostatic immunoglobulin levels, with the exception of IgA in *april*$^{-/-}$ mice (74). In contrast, *baff*$^{-/-}$*april*$^{-/-}$ mice on an autoimmune NZM background have fewer bone marrow plasma cells and autoantibodies than *baff*$^{-/-}$ NZM mice. Atacicept is an Fc fusion protein fused to the extracellular domain of TACI and binds BAFF, APRIL, and BAFF/APRIL heteromers (76). Treatment with atacicept in SLE patients resulted in rapid decreases in serum IgM (~70%), IgG (~30–40%), and IgA (~50–60%) as well as ~40% decreases in anti-dsDNA antibodies. Antibody and autoantibody levels returned to pretreatment levels following discontinuation of therapy. In a phase 2 clinical trial, atacicept did not demonstrate efficacy in extrarenal lupus, though a trend toward efficacy was observed in patients with high disease activity treated with the higher 150-mg subcutaneous weekly dose (76). At that dose, patients demonstrated improvement in flare rates and time to flare, though this treatment arm was discontinued due to two patient deaths (77). Both patients were treated with atacicept. The first was a 22-year-old male with SLE and overlap syndrome features with scleroderma who developed acute respiratory failure and alveolar hemorrhage possibly due to leptospirosis. The second was a 30-year-old female who died due to pneumococcal pneumonia and alveolar hemorrhage secondary to SLE. Atacicept was also tested in multiple sclerosis (MS), but it was discontinued as it worsened RMS disease activity (78) (see below).

Integrins

Integrins regulate immune cell trafficking by modulating leukocyte adhesion to blood vessels and facilitate their extravasation into tissues. Integrin heterodimers consist of pairings between one each of 18 α subunits and 8 β subunits forming 24 different receptor complexes that differ in expression patterns, ligand specificity, and function (79, 80). Genetic mutations in *ITGB2* resulting in absent or reduced expression of the β_2 integrin (CD18) compromise expression of $\alpha_L\beta_2$ (CD11a/CD18, LFA-1), $\alpha_L\beta_2$ (CD11b/CD18, Mac-1, or CR3), $\alpha_X\beta_2$ (CD11c/CD18, p150/95, or CR4), and $\alpha_D\beta_2$ (CD11d/CD18) to cause leukocyte adhesion deficiency I (LAD-I). Patients with LAD-I are susceptible to bacterial and fungal infections affecting skin and mucous membranes. Since leukocytes are unable to extravasate into tissue, a hallmark of LAD-I is the inability to form pus at sites of infection. In addition to host defense, integrins also contribute to the trafficking of leukocytes to promote inflammatory and autoimmune responses. Expression of integrins and their corresponding ligands are elevated in many diseases.

In MS, $\alpha_4\beta_1$ (CD49d/CD29, VLA4) is expressed on T and B cells and facilitates lymphocyte extravasation into the central nervous system (CNS) through binding of VCAM1 on blood-brain barrier endothelial cells. In the gastrointestinal tract, $\alpha_4\beta_7$ expressed on T cells can bind MADCAM-1 on Peyer's patch high endothelial venules and lamina propria venules to facilitate extravasation of pathogenic T cells in inflammatory bowel diseases (IBD). Natalizumab is an α_4 blocking mAb approved for treatment of patients with CD and RMS (81, 82). However, the mechanistic basis for efficacy in RMS of inhibiting lymphocyte trafficking into the CNS also provides the basis for development of infrequent cases of progressive multifocal leukoencephalopathy (PML), a devastating demyelinating disease caused by the John Cunningham virus (83). A second generation of antibodies, including vedolizumab (selective for the $\alpha_4\beta_7$ complex) and etrolizumab (anti β_7),

reduce the likelihood of PML by targeting gut-specific integrins for IBD. Vedolizumab induces clinical response and remission and is FDA approved for both ulcerative colitis (UC) and CD (84, 85). In addition to blocking $\alpha_4\beta_7$:MADCAM1 interactions, etrolizumab interferes with the retention of $\alpha_E\beta_7^+$ IELs by E-cadherin$^+$ intestinal epithelial cells. In a phase 2 clinical trial, treatment with etrolizumab in patients with UC resulted in greater likelihood of clinical remission when compared to control treatment (86). As $\alpha_E\beta_7^+$ IELs are significant contributors of inflammatory cytokines in patients with UC, additional clinical benefit may be possible by blockade of both $\alpha_4\beta_7$ and $\alpha_E\beta_7$ integrins (87). Data from ongoing phase 3 clinical trials and longer-term follow-up will be required to better understand both efficacy and safety.

Efalizumab, an anti α_L antibody, blocks the interaction of $\alpha_L\beta_2$ (LFA1) expressed on T and B cells with ICAM1. In addition to blocking integrin, efalizumab also downregulates LFA-1 expression, which is required for proper formation of the T cell synapse with antigen-presenting cells (APCs). In turn, T cell receptor–mediated activation is compromised. Efalizumab was FDA approved for treatment of moderate to severe plaque psoriasis. However, four patients treated with efalizumab for more than three years developed PML, and the drug was voluntarily withdrawn from the market (88).

The experience with integrin immunodeficiency and therapeutic blockade underscores the central roles of this family of adhesion and signaling proteins in host immunity and inflammatory diseases. Organ-specific delivery of therapeutics to diseased tissue while sparing systemic inhibition may further increase the therapeutic potential for integrin targeting agents. As one example, lifitegrast, an LFA-1/ICAM-1 small-molecule inhibitor is approved for treatment of dry eye disease (DED). DED is characterized by localized inflammation of the ocular surface and periocular tissues with homing of activated T cells, inflammatory cytokines, and hyperosmolar tears that results in eye dryness and discomfort. Topical application of lifitegrast as an ophthalmologic solution provides improvement in inferior corneal staining score and eye dryness, particularly in patients with moderate to severe symptoms (89). Consistent with the minimal systemic absorption and rapid hepatic clearance, no systemic adverse events have been associated with ophthalmic instillation of lifitegrast. Development of drug delivery strategies to localize inhibitory activity will permit greater therapeutic opportunities for integrin targeting.

Interleukin-12 and -23 and Downstream Effectors

IL-12 and IL-23 are two closely related, APC-derived, heterodimeric cytokines consisting of p35 and p19 subunits, respectively, that pair with a common p40 subunit (90). They bind to heterodimeric receptors with private IL-12Rβ2 (IL-12) and IL-23R (IL-23) subunits that pair with a common IL-12Rβ1 transmembrane receptor. p40 can also exist as a homodimer ($p40_2$), the function of which remains unclear. IL-12 and IL-23 production is elicited through TLR and dectin pathway activation of APCs. For either cytokine, the main responder cells are T and innate lymphoid cells (ILCs), although responses in other immune cell types have also been described. Across cell types, IL-12 upregulates expression of the Th1 master transcription factor T-bet and induces production of IFN-γ. In turn, IFN-γ acts on myeloid cells to combat intracellular pathogens, such as mycobacteria or toxoplasma. Genetic defects compromising production of IL-12 or IFN-γ are associated with mycobacterial infections, though immune responses to viruses, fungi, and other pathogens remain intact (91). In an analogous fashion, IL-23 stimulation induces the expression of the transcription factor RORγt and expression of the downstream cytokines IL-17A, IL-17F, IL-22, and GM-CSF (92). The major role of IL-17 is to recruit neutrophils and protect against extracellular bacteria and fungi. IL-22 signaling to epithelial cells results in production of antimicrobial peptides and enhances mucosal barrier function. Thus, the main functions of IL-23 are

maintaining barrier function and neutralizing extracellular pathogens. Consistent with the central role of IL-12 and IL-23 in host mucosal defense, preclinical models of disease and genome-wide association studies implicate IL-12/23 pathway in inflammatory bowel diseases (93) and psoriasis (94).

Multiple therapies designed to interfere with various components of IL-12/23 pathways have been advanced into the clinic (**Table 1**). Two mAbs (ustekinumab and briakinumab) target the p40 subunit and thus neutralize both IL-12 and IL-23. More recently, five mAbs targeting IL-23 selectively have been introduced. The IL-17 family of effector cytokines have attracted much attention with five mAbs that selectively neutralize IL-17A; two that neutralize IL-17A and F; and brodalumab, which targets IL-17RA, a common receptor for IL-17 A, B, C, E, and F members. However, while distinct functions have been described for IL-17B–E (95), these cytokines are not regulated by IL-23 and their roles in human disease remain unexplored. Finally, studies with therapies blocking IL-22 (fezakinumab), IFN-γ (fontolizumab), and GM-CSF (namilumab and mavrilimumab) will provide further insights, though these are much earlier in their development.

INSIGHTS INTO HUMAN DISEASE BIOLOGY ACROSS THERAPEUTIC CLASSES

Psoriasis

Chronic plaque-type psoriasis (psoriasis vulgaris) is the most common form of psoriasis and manifests as erythematous plaques with thick scaling occurring anywhere on the body. Symptoms include itching, bleeding, and pain; furthermore, the disease can lead to disfiguration and considerable psychological burden. Skin lesions are characterized by parakeratosis and thickened projections of the prickle cell layer of keratinocytes (psoriasiform hyperplasia). Polymorphonuclear leukocytes and lymphocytes infiltrate both dermis ($CD8^+$) and epidermis ($CD4^+$). PsA and CD often coexist in patients with psoriasis (96, 97). The most widely used tool for the measurement of disease activity is the Psoriasis Area and Severity Index (PASI), which combines assessment of severity and area affected into a single score ranging from 0 (no disease) to 72 (maximal disease) (98).

The pathophysiology of psoriasis is complex and, to some extent, predicated on the genetic makeup of the host. There is a strong association between streptococcal infection and psoriasis (99). A well-characterized genetic risk factor is HLA-C:06, which likely gives rise to autoreactive $CD8^+$ T cells due to its propensity to present streptococcal protein fragments that share extensive sequence homology with epidermal keratins (100). Additional genetic loci, including components of the IL-23 and NF-κB signaling systems, have been identified and collectively account for ∼28% of the genetic heritability (101).

A pathogenic role for T cells is supported with clinical efficacy with several T cell–targeted therapies, namely abatacept (102), alefacept (103), and efalizumab (104). Furthermore, TNF antagonists are approved by the FDA for the treatment of psoriasis (**Table 1**). We refer readers elsewhere for reviews on these earlier discoveries (105). The discovery of Th17 cells (106) and the subsequent finding that IL-17-producing $CD4^+$, $CD8^+$, ILC, and γδ T cells are prominently present in psoriatic skin lesions have recently focused treatment approaches on the IL-23/17 axis (107).

In two pivotal clinical trials with anti-IL-12/23 agents, ustekinumab achieved PASI 75 scores of 66.4–75.7% after 12 weeks of treatment (108, 109). Direct comparisons enabled by head-to-head trials demonstrated superiority of ustekinumab to the TNF antagonist, etanercept (56.8% achieved PASI 75) (110). Similarly, efficacy and superiority over etanercept were achieved with

briakinumab (111–113). While children with genetic defects in the IL-12 pathway experience mycobacterial infections (114), no major infections have been observed in an integrated analysis of 5,884 ustekinumab-treated patients over 4,521 patient-years of exposure (115). Less is known about the safety of briakinumab, as it was withdrawn from clinical development. An imbalance of seven malignancies in briakinumab-treated patients was observed across three randomized, controlled trials (111–113).

IL-23 blockade with risankizumab, guselkumab, and tildrakizumab also provided clinical benefit and superiority to TNF inhibition in patients with psoriasis (115–118). Interestingly, a subgroup analysis of the two guselkumab trials revealed that its superiority to adalimumab was primarily derived from disease resolution on the scalp, palms, and soles, while the magnitude of improvement in fingernails did not differ between treatments (119). While this finding needs to be reproduced, it is tempting to speculate that differential commensal colonization of various skin sites may hold clues to the mechanistic understanding of this difference (120). Surprisingly, IL-23 selective antibodies outperformed ustekinumab, which neutralizes both IL-12 and IL-23. Several hypotheses are available to explain this unexpected finding. The first is simply that ustekinumab was not dosed high enough. p40 is found at ~100 pg/mL in the serum of psoriatic patients (121), whereas IL-23 levels are >10-fold lower (122), suggesting that saturation of the therapeutic mAb is more likely to occur for ustekinumab than for any of the IL-23 selective agents. In support of this hypothesis, several trials indicate that a plateau has not been reached with ustekinumab, and lowering the dose led to a drop in efficacy. However, there is insufficient published analysis of pharmacokinetic and pharmacodynamic parameters to definitively corroborate or reject this hypothesis. A second possibility is that IL-12 provides a protective effect in psoriasis. IL-12 has been demonstrated to restrain the influx of IL-17-producing Vγ6Vδ1 T cells into inflamed skin as well as suppress IL-17 production in Th17 cells (123, 124). In addition, IL-12 promotes a protective transcriptional program to limit TNF-mediated inflammation. Finally, ustekinumab, but presumably not anti-p19 antibodies, will inhibit $(p40)_2$, which has been demonstrated to function as both an antagonist of IL-12/23 signaling and an agonist (125).

The major effector cytokines downstream of IL-23 include IL-17, IL-22, and GM-CSF. Namilumab, a GM-CSF antibody, did not provide clinical benefit in psoriasis (126). Serum levels of IL-17 and IL-22 levels are both elevated in psoriasis patients, and treatment with etanercept results in a reduction in both cytokines. However, only IL-17 levels correlated with PASI scores (127). Two IL-17A selective antibodies, secukinumab (128–130) and ixekizumab (131), are similarly efficacious as anti-IL-23 mAbs, and superior to ustekinumab (128, 129, 132) and etanercept (130, 133). Brodalumab (134, 135) and bimekizumab (136), targeting IL-17RA and IL-17A/F, respectively, have also demonstrated clinical efficacy. Due to the lack of head-to-head studies, it is currently not possible to determine whether neutralization of additional IL-17 family members by these latter reagents confers additional benefit. Most recently, a head-to-head study of guselkumab versus secukinumab suggested that IL-17 inhibition may not be quite as effective as IL-23 blockade (117), though these results need replication. IL-17 blockade has thus far shown a favorable safety profile in psoriasis, though a small increase (1.7–4.0%) in the frequency of *Candida* infections has been observed (137).

PsA additionally involves joint and tendon inflammation (96). A head-to-head study of ixekizumab against adalimumab confirmed the superiority of IL-17 blockade with regard to skin involvement. In addition, IL-17 blockade also demonstrated efficacy in improvement of PsA, including inhibition of radiographic progression supporting the ability of IL-17 targeting to have disease-modifying effects (138). Furthermore, a bispecific antibody that neutralizes both IL-17 and TNF-α (remtolumab) failed to demonstrate superiority over adalimumab (TNF-α) in PsA, suggesting that any patients that might respond to IL-17 blockade are already included in

anti-TNF-α responders. These results highlight intradisease heterogeneity with different clinical manifestations being differentially susceptible to therapeutic intervention.

Inflammatory Bowel Diseases

CD and UC are the two dominant forms of IBD (139, 140). Disease results from dysregulated inflammatory responses to intestinal microbes in genetically susceptible hosts with compromised mucosal barrier functions, autophagy pathways, and Th17 biology (141). The two diseases differ in anatomical location, histology, risk factors, and comorbidities. Efficacious therapies approved by the FDA, to date, include TNF-α antagonists, vedolizumab and natalizumab, and JAK inhibition, with tofacitinib approved for UC.

Ustekinumab is FDA approved for treatment of CD (142) and UC (143, 144). Efficacy of IL-23-targeting agents, brazikumab (145) and risankizumab (146), has also been reported in phase 2 clinical trials. Whereas IL-12/23- and IL-17-targeting mAbs have demonstrated concordant efficacy in psoriasis, they demonstrate discordant clinical results in CD. Neither secukinumab (147) nor brodalumab (148) provided benefit in CD. Brodalumab led to worsening of CD, and secukinumab demonstrated a similar trend, though the study was underpowered to detect statistically significant worsening. The lack of efficacy or CD worsening may be due to a homeostatic role of IL-17 in the maintenance of intestinal epithelial tight junctions (149). While treatment with IL-17-targeting therapies does not cause IBD in patients with psoriasis or spondyloarthropathy, a small number of de novo cases of IBD have been observed in multiple non-IBD trials with different IL-17-blocking agents (131, 150–156).

Multiple Sclerosis

MS is a chronic autoimmune disorder initiated by immune cell infiltration across a compromised blood-brain barrier to promote CNS inflammation, gliosis, axonal demyelination, and oligodendrocyte loss (157, 158). About 85% of patients have RMS with loss in both physical and cognitive functions over time. The remaining 15% of patients suffer from primary progressive MS (PPMS) with steady deterioration of neurologic function. Clinical symptoms are defined by the anatomic location of the inflammatory and neurodegenerative process. Inflammatory lesions involve both adaptive and innate immune cells including CNS-resident microglial cells.

The importance of immunity in MS is supported by strong genetic association with the HLA-DRB1 locus ($P < 10^{-23}$) and other immune-relevant genetic loci (e.g., IL-2 and CD25) (159). Therapies in RMS have focused on immunomodulation. Consistent with the genetic studies implicating MHC class II molecules in disease risk, T cell–directed therapies have demonstrated clinical benefit. Similarly, the benefit of ocrelizumab is likely due to contributions of B cells as APCs, providing costimulatory signals and secreting proinflammatory cytokines (57). Underscoring differences in immune contributions in RMS relative to PPMS, all immunomodulatory therapies tested to date have failed in providing clinical benefit in PPMS, with the exception of ocrelizumab. The neurodegenerative component in PPMS appears to be more dominant than the immune component, as patients treated with ocrelizumab, while experiencing less decrement in their disability, still experience significant deterioration despite the absence of new inflammatory CNS lesions as assessed by magnetic resonance imaging (MRI) (160). Future drug discovery focused on neuronal regeneration, remyelination, and enhancing regulatory immune cells may provide additional clinical benefit.

Two additional insights were derived from the successes and failures in MS across different classes of immunomodulatory therapies.

1. Discordance between IL-12/23- and IL-17-targeted therapies. While IL-23 is required for development of Th17 cells, there is a discordance in the therapeutic response of anti-IL-12/23 and anti-IL17A therapies. In a phase 2 trial in RMS, secukinumab decreased the number of gadolinium (Gd)-enhancing MRI brain lesions (161). In contrast, ustekinumab provided no improvement in Gd-enhancing lesions (162). Preclinical studies using experimental autoimmune encephalitis (EAE), as a model of MS, indicate that IL-23 is important for disease initiation, but once primed, IL-23 is dispensable for the effector disease phase in EAE (163).
2. B cell modulation. While anti-CD20 mAb therapy is effective in RMS, treatment with atacicept (TACI-Fc) that blocks both BAFF and APRIL increased clinical relapses (78). The detrimental effects observed with atacicept may be due to the role of BAFF/APRIL in maintaining immunosuppressive IL-10-producing B cells (164).

Type 2 Immune Diseases

Type 2 immunity evolved to rid hosts of extracellular parasites and is orchestrated by cytokines originally attributed to Th2 cells (IL-4, IL-5, IL-9, and IL-13) (165). Studies over the last decade have revealed that type 2 cytokines, particularly IL-5 and IL-13, are also produced by group 2 ILCs (ILC2s), mast cells, basophils, eosinophils, $CD8^+$ T cells, and natural killer T cells (166). These cytokines promote eosinophil differentiation and survival (IL-5); eosinophil tissue homing (IL-13); B cell class switching to IgE production (IL-4 and IL-13); goblet cell differentiation and mucus production (IL-13); smooth muscle hyperplasia and contractility (IL-13); and mast cell differentiation, activation (IL-9), and degranulation (via IgE-antigen complex cross-linking of FcεRI).

Epithelial injury, caused by pathogens or environmental factors, elicits the release of alarmins, including IL-33, IL-25, IL-1α, and thymic stromal lymphopoietin (TSLP), to act on innate tissue-resident cells including ILC2s, mast cells, and dendritic cells to rapidly mobilize a type 2 cytokine response aimed at neutralizing, killing, and expelling multicellular parasites (167). The dramatic rise in allergic and autoimmune diseases over the last several decades may be explained by the hygiene hypothesis, which posits that in clean modern societies, insufficient exposure to non-pathogenic commensal bacteria in early life results in subsequent inappropriate immune responses to what should be harmless environmental antigens, such as dust mites, pet dander, molds, and pollen (168). Several disorders appear to be due to dysfunction of type 2 immunity, as indicated by efficacious strategies to perturb this pathway in the clinic.

Asthma heterogeneity. Asthma is a common respiratory disorder characterized by reversible, episodic airway obstruction and hyperreactivity. Symptoms include expiratory wheeze, cough, nighttime awakenings, and compromised exercise capacity. In some asthma patients, acute exacerbations characterized by worsening of symptoms and a precipitous decline in lung function may occur (169). Asthma often develops in childhood, particularly in children with atopy who experience a lower respiratory viral infection prior to the age of two years (170). However, it may also develop later in life and often presents without evidence of allergic inflammation (171). Triggers include indoor and outdoor aeroallergens; cigarette smoke; pollutants such as ozone; and infectious agents including viruses, bacteria, and fungi.

In the majority of patients, asthma is adequately controlled with short- or long-acting β-adrenergic agonists (SABA or LABA) for symptomatic relief of wheeze and airway obstruction and with inhaled corticosteroids (ICS) and/or oral leukotriene receptor antagonists as a prophylactic anti-inflammatory strategy to prevent episodic worsening of symptoms. Up to about 10%

of patients have poor asthma control despite high-dose LABA/ICS treatment, require frequent systemic corticosteroids, have a high symptom burden and frequent exacerbations, and consume over half of the health care expenditures on asthma (172, 173). This subset of severe-asthma patients represents a large unmet medical need and has been the focus of new biologic therapies targeting inflammatory mediators (174).

Patients with severe asthma fall into two categories depending on the presence or absence of elevated numbers of eosinophils in airway tissue. Eosinophilic asthma patients have higher numbers of other inflammatory cells (including mast cells) and increased bronchial fibrosis and are more likely to require mechanical ventilation. However, they do not differ from the noneosinophilic patients in lung function, airway hyperresponsiveness, or clinical symptoms (175). Molecular characterization of bronchial epithelium revealed an IL-13-inducible gene signature in approximately half of mild- to moderate-asthma patients. This type 2 high gene signature largely identified eosinophilic asthma patients (176). Thus, a hypothesis emerged that therapies targeting type 2 inflammatory pathways would be more likely to provide benefit in type 2–high than in type 2–low asthma patients. Several noninvasive biomarkers can distinguish these phenotypes, including measurements of sputum and blood eosinophils, fractional exhaled nitric oxide (FeNO), and serum or plasma levels of soluble IL-13-inducible proteins secreted by bronchial epithelium such as periostin (177). Although these biomarkers are useful to stratify asthma patients, it is important to recognize that they are all continuously distributed across populations and do not identify discrete subsets. Different studies have used different assays and cutoffs, hampering the ability to draw direct comparisons between studies in many cases (178).

Biologic therapies targeting type 2 mediators in asthma that have progressed through phase 2 proof-of-concept and phase 3 pivotal studies include omalizumab (anti-IgE), mepolizumab and reslizumab (anti-IL-5), benralizumab (anti-IL-5Rα), lebrikizumab and tralokinumab (anti-IL-13), and dupilumab (anti-IL-4Rα, a receptor for both IL-4 and IL-13) (169) (**Table 1**). Collectively, these studies have confirmed that asthma is heterogeneous with respect to type 2 inflammation, necessitating biomarker-guided patient stratification (**Table 2**). Furthermore, outcome measures such as lung function, symptoms, and exacerbation rates do not strongly correlate with each other, suggesting that these clinical manifestations may have different underlying causes and thus respond differentially to a given intervention. Finally, seasonality was observed in multiple trials for the exacerbation end point, with rates being highest in the spring (likely due to pollen aeroallergens) and in the fall (increased transmission of respiratory viruses) (179–181).

IgE-targeting therapies. Omalizumab binds to the Fc region of serum IgE and reduces free circulating IgE by ∼95%. As occupancy of FcεRI by IgE on basophils and mast cells is required to maintain its plasma membrane expression, surface FcεRI expression is diminished by ∼90% following omalizumab therapy (182). In a bronchial allergen challenge study in subjects with mild allergic asthma, omalizumab reduced both early-phase and late-phase allergen responses (183). The early-phase response beginning within the first hour of allergen challenge is primarily due to inflammatory mediators (e.g., histamine and leukotrienes) released from mast cells and causes smooth muscle contraction, airway edema, and mucus secretion. The late-phase response occurs three to eight hours after challenge and is driven by continued inflammation and infiltration of eosinophils and mast cells (184). In pivotal clinical trials, patients treated with omalizumab experienced significantly fewer asthma exacerbations than those receiving placebo, but omalizumab treatment did not prevent all exacerbations (185). In pediatric asthma patients, omalizumab was most effective in preventing seasonal spikes in exacerbation rates, with the greatest reduction relative to placebo observed in spring and autumn months (180). Patients with elevated levels of type 2 biomarkers including blood eosinophils, FeNO, or serum periostin experienced the

greatest benefit in terms of exacerbation reduction (186); as was the case for seasonal exacerbations, this reduction was a consequence of generally higher background exacerbation rates in the placebo arms for type 2–high patients as compared to type 2–low patients. Omalizumab provides modest benefit on other outcome measures including lung function and symptoms (185); thus, its main benefit appears to be in terms of preventing seasonal exacerbations in type 2–high patients with evidence of underlying atopy.

As B cells express transmembrane IgE prior to differentiating into IgE-secreting plasma cells, quilizumab, an afucosylated antibody (to enhance FcγR-mediated depletion) against a unique epitope of transmembrane IgE that is not encoded in secreted IgE, was tested to determine whether depletion of IgE-switched B cell precursors could eliminate the allergic B cell repertoire. While quilizumab treatment attenuated both early- and late-phase allergen responses and induction of newly synthesized allergen-specific IgE in a bronchial allergen challenge study (187), it did not prevent exacerbations in a larger study of moderate- to severe-asthma patients (188). In that study, quilizumab reduced serum IgE levels by only about 25% over a nine-month period, suggesting that the half-life of IgE-producing plasma cells is substantially longer, likely on the timescale of years.

IL-5-targeting therapies. Three mAb therapies targeting IL-5 have been approved for the treatment of asthma (**Table 1**): Mepolizumab and reslizumab inhibit IL-5 binding to its receptor, whereas benralizumab, an afucosylated antibody against IL-5Rα, additionally depletes IL-5Rα-expressing cells, including eosinophils and basophils (174).

Studies of mepolizumab in severe-asthma patients with evidence of airway, blood, and sputum eosinophilia and a history of frequent exacerbations demonstrated that IL-5 inhibition resulted in substantial reductions in circulating eosinophils, asthma exacerbations, and oral steroid use. However, there were only modest improvements in lung function and chronic symptom burden (189). In addition, anti-IL-5 therapies have little effect on circulating IgE levels or FeNO (190). Studies with reslizumab and benralizumab subsequently demonstrated similar outcomes, albeit with subtle differences in enrollment requirements and stratification variables (i.e., blood eosinophil level cutoffs) (174). Thus, these studies revealed a central role of IL-5 in driving eosinophilic inflammation, and by extension, the role of eosinophilic airway inflammation in contributing to some, but not all asthma exacerbations in type 2–high asthma patients. Meanwhile, the effects on baseline lung function and day-to-day symptoms between exacerbations were modest, suggesting different underlying mechanisms.

As an interesting vignette, an early study with mepolizumab in mild allergic asthma patients failed to demonstrate any effect on early- or late-phase allergen responses, suggesting that eosinophils are not a significant component of the allergen response (191). The benefit of anti-IL-5 therapies in reducing spontaneous exacerbation rates in pivotal outcome trials therefore calls into question the translatability of the bronchial allergen challenge study design to more real-world settings and suggests that clinical end points must be carefully matched to the disease biology targeted by the intervention.

IL-13/IL-4-targeting therapies. Several antibodies targeting the IL-4/13 pathway, including lebrikizumab, tralokinumab, and dupilumab have progressed through pivotal trials. Agents targeting IL-4 alone (pascolizumab, altrakincept) failed to demonstrate benefit, suggesting that IL-13 is the key effector cytokine of the two in asthma (192). Lebrikizumab demonstrated benefit in mild type 2–high asthma patients in a small bronchial allergen challenge study, reducing late-phase, but not early-phase allergen responses (193). Such a result implicates IL-13 in the recruitment of inflammatory cells secondary to IgE-allergen complex–mediated mast cell degranulation. Thus,

anti-IL-13 is distinguished from anti-IL-5, which had no effect on bronchial allergen responses (191), and anti-IgE, which prevented both early-phase and late-phase responses (183).

Although dupilumab, lebrikizumab, and tralokinumab all exhibited efficacy in phase 2 trials (194–199), dupilumab emerged as a superior therapy in pivotal year-long phase 3 trials (200). Whereas both lebrikizumab and tralokinumab achieved modest exacerbation rate reductions and lebrikizumab substantially improved lung function, neither met its prespecified primary end point of exacerbation rate reduction in replicate phase 3 trials (201, 202). By contrast, dupilumab achieved significant lung function improvement and exacerbation reduction in type 2–high patients (200). Consistent with IgE- and IL-5-targeting therapies, a post hoc analysis of the lebrikizumab studies showed that it prevented seasonal exacerbation rate spikes in type 2–high patients (179).

The divergent clinical experience with IL-13-targeting therapies illustrates the difficulty in interpreting clinical trial results. In alignment with our considerations on this topic, there are several potential explanations for the discrepant trial results (203):

1. Target selection. Dupilumab inhibits both IL-4 and IL-13 signaling, and although targeting IL-4 selectively provided no discernible therapeutic benefit (192), it remains a possibility that IL-4 becomes important when IL-13 is neutralized. The pharmacodynamic biomarker effects on FeNO, IgE, and blood eosinophils primarily reflected IL-13 biology and were thus concordant across agents. There was no assessment of—nor clear evidence for—IL-4 selective biomarkers.
2. Molecular properties. While there may be subtle molecular differences, dupilumab constitutively occupies the receptor. By contrast, IL-13-targeting agents may be more dependent on binding affinities and ability to neutralize ligand in all microenvironments and spatial configurations relative to its receptor (204).
3. Study patient population. Because the lebrikizumab trials did not require recent exacerbations as an enrollment criterion, the placebo arm exacerbation rate was low (less than one patient per year). Over 60% of patients in the placebo arms had zero exacerbations, rendering them uninformative with respect to the primary outcome measure (201). In contrast, dupilumab studies (200), similar to those of mepolizumab and benralizumab, required a history of exacerbations in the prior year, which led to a higher exacerbation rate in the trial and thus a larger window to demonstrate statistically significant reductions.
4. Study patient stratification. The pivotal lebrikizumab studies prespecified a composite of serum periostin and blood eosinophils that classified 70% of all patients as biomarker-positive; one of the replicate studies met its primary outcome measure in this subset with statistical significance while the other did not (201). The dupilumab studies used biomarker stratification as a secondary outcome measure and showed consistent exacerbation benefit primarily in patients with >300 eosinophils/mm^3 of blood, a subset of only $\sim$45% of patients (200). Thus, as these type 2 biomarkers are continuously distributed across patients, using more stringent definitions of type 2–high can yield better outcomes for type 2 cytokine inhibitors.

Overall, studies of IgE, IL-5, and IL-4/13 inhibitors have been remarkably informative about the roles of type 2 inflammation in human asthma patients. About half of severe asthma exacerbations can be prevented, particularly seasonal spikes in patients with high levels of type 2 inflammation. While IgE and IL-5 inhibition do not consistently impact lung function across the populations examined, IL-13 inhibition improves lung function when administered on top of background ICS therapy, which may reflect the direct effects of IL-13 on mucus production and composition that contribute to small-airway obstruction (169). Nevertheless, none of the

efficacious type 2 cytokine–targeted therapies completely prevent asthma exacerbations or eliminate day-to-day symptoms, particularly in type 2–low patients. Thus, the unmet need in asthma has been redefined with a remaining key focus on type 2–independent pathways.

Type 2–independent immunity in asthma. The success of biological therapies targeting type 2–high asthma patients highlights the residual unmet medical need in patients without elevated type 2 airway inflammation. While several hypotheses for pathophysiological mechanisms have been postulated, no therapy has yet been approved specifically for the type 2–low patient subset. Tezepelumab, a mAb directed against TSLP, is currently being investigated in phase 3 trials. In a phase 2b study, it reduced exacerbation rates in both type 2–low and type 2–high patients, and provided lung function benefits predominantly in type 2–high patients (205). In stable severe asthma, a subset of patients with elevated airway neutrophils and eosinophils (a mixed granulocytic phenotype) is associated with elevations in both type 2 cytokines and Th17 cytokines as well as decreased responsiveness to ICS (206); other studies have implicated elevated levels of innate cytokines: e.g., IL-6 in obese severe-asthma patients (207), type I and type II interferons (208, 209), and altered bacterial flora in the airway (210). Thus far, trials with TNF-α and IL-17 inhibitors have failed to yield positive outcomes in asthma patients (54, 211), although those studies did not use biomarkers to select patients on the basis of evidence for activity of those pathways; hence, an effect in a small subset may have been missed. Overall, despite the considerable benefit provided by asthma therapies targeting IgE, IL-5, and IL-4Rα, the inability of these agents to consistently improve lung function and symptoms or completely prevent asthma exacerbations, seasonal variability in clinical presentation, and the identification of biologically distinct subclusters of asthma exacerbations (212) define the residual unmet need and identify avenues for future investigation.

Type 2 immunity beyond asthma. Disease mechanisms present in asthma are also active in other indications, and accordingly, these biotherapeutics have yielded new insight and treatment options for other indications:

1. Atopic dermatitis (AD) is a chronic inflammatory skin disorder characterized by epithelial barrier dysfunction, eczematous lesions, and high levels of IL-13, IgE, and eosinophilic infiltration. It can be exacerbated by an itch-scratch cycle, in which bacterial skin flora are introduced into the dermis, promoting further inflammation (213). Dupilumab is efficacious and is the first biologic therapy approved for AD (214). Lebrikizumab and tralokinumab have also shown activity in AD (215, 216).
2. Chronic spontaneous urticaria (CSU) is a dermatologic condition due to autoantibodies against FcεRIα and IgE-isotype autoantibodies, with frequent and inappropriate mast cell degranulation giving rise to hives (217). Omalizumab is the first therapeutic to demonstrate significant benefit beyond symptomatic treatment with antihistamines in CSU, underscoring a role for IgE-FcεRI interactions in CSU pathogenesis (218).
3. Rare eosinophilic disorders include hypereosinophilic syndrome (HES), a systemic disorder characterized by very high levels of circulating eosinophils and tissue infiltration including skin, gastrointestinal tract, and lungs with activated eosinophils; and eosinophilic granulomatosis with polyangiitis (EGPA, also known as Churg-Strauss syndrome), an autoimmune disorder with eosinophil-mediated vasculitis (219). Mepolizumab and other IL-5 inhibitors have demonstrated efficacy in HES, reducing eosinophilic infiltration and symptoms by more than half in most patients (220); mepolizumab is approved for treatment of patients with EGPA (221).

4. Despite the presence of a type 2 signature and elevated eosinophil levels in a subset of patients suffering from chronic obstructive pulmonary disease (COPD), an airway disorder common in smokers, neither IL-5- nor IL-13-targeting therapies have demonstrated consistent significant benefit in COPD, even in a subset with elevated eosinophils (222). It is likely that these negative results reflect a relative lack of contribution of type 2 cytokines to COPD pathogenesis, because the same therapies, at the same dosages, are active in asthma patients.

Taken together, these studies across multiple indications show varied roles for type 2 mediators in multiple systemic disorders and underscore the importance of carefully linking molecular target activity to pathological manifestations and clinical outcome measures.

Rheumatoid Arthritis

RA is a chronic autoimmune inflammatory disorder primarily affecting joints, but it can also have systemic extra-articular manifestations (223). It is a diagnosis of exclusion and is defined by a set of diagnostic criteria that includes the number of joints affected; duration of symptoms; presence of rheumatoid factor (RF) or anticitrullinated peptide antibodies; and evidence of abnormal levels of acute-phase reactants, CRP, or erythrocyte sedimentation rate. A standard measure of therapeutic benefit in clinical trials is through the use of the American College of Rheumatology (ACR) criteria, an aggregate assessment of physical findings, measurements of acute phase reactants, as well as patient and physician assessments. ACR20, 50, and 70 indicate 20%, 50%, and 70% improvement, respectively.

Therapeutics targeting five biological target classes have been approved by the FDA for treatment of various patient subsets with RA (**Table 1**). Despite the availability of this armamentarium of therapeutics, the placebo-corrected ACR50 scores range from 11% to 33% for treatment of RA patients who have failed a disease-modifying antirheumatic drug, such as methotrexate. That no single class of therapeutics can provide >33% improvement in their placebo-corrected ACR50 scores supports the notion that RA represents a heterogeneous clinical syndrome, in which different immune mechanisms lead to a common clinical presentation.

In support of this hypothesis, three patterns of synovial pathologies were initially described in 1984: (*a*) abundant lymphoid infiltrates with GCs, (*b*) abundant lymphoid infiltrates without GCs, and (*c*) fibroblastic/synovial proliferation with few lymphoid cells (224). More recently, and with the help of molecular techniques and immunohistochemical reagents, three synovial pathotypes have been defined based on histology and gene signatures: lymphomyeloid, diffuse myeloid, and pauci-immune. All three synovial pathotypes are observed in both early (mean duration of less than six months) and late (mean duration of more than three years) disease (225, 226). The lymphomyeloid pathotype is defined histologically by presence of T cells, B cells, plasma cells, and myeloid cells and molecularly by a high lymphoid gene expression score. GC-like structures are most commonly detected in the lymphomyeloid pathotype. These patients tend to respond better to tocilizumab or B cell depletion. The diffuse myeloid pathotype is histologically and molecularly characterized by a myeloid lineage predominance. These patients have a preferential response to TNF-α-targeting agents. The pauci-immune pathotype is histologically characterized by fibroblasts with rare immune cells. This pathotype is associated with lower acute phase reactants and serologies and tends to spare larger joints (e.g., knees) but still has evidence of significant disease activity. Of note, the lymphoid gene signature score is associated with faster radiographic destruction; accordingly, an osteoclast gene signature is correlated with this pathotype. These pathotypes underscore the heterogeneity of patients with RA and the need for further determination of optimal therapies for each synovial pathotype to improve patient responses.

WHAT'S NEXT?

Significant benefit to patients and important insights into basic immunology and disease pathogenesis have been derived from development of biotherapeutics over the last more than three decades. These clinical experiments have also revealed significant challenges to new therapeutic development due, in part, to disease heterogeneity and clinical study design including patient selection and end point selection as well as biomarker discovery and development. While significant unmet medical needs remain for new drug discovery efforts, the tools and approaches used for new target discovery and clinical candidate generation to address these challenges continue to grow. Some of these exciting developments include the following:

1. Human genetics for target discovery. Lowered costs of DNA sequencing have enabled new applications of human genetics for target discovery. Modifier screens for genetic elements that alter disease severity may provide clues for therapeutic targets. For example, IL-6R was identified as a candidate modifier of age of onset in Alzheimer disease (227). Search for genetic risk factors for disease progression, rather than disease development, may reveal novel pathways, as has been described for CD (228). In addition, identification of rare loss- or gain-of-function variants may provide important insights into common disease (229): The discovery of African Americans with nonsense mutations in *PCSK9* associated with reductions in LDL and protection against cardiovascular disease (230) provided the basis for PCSK9 antagonists.
2. Novel drug platforms. While most biotherapeutics to date have focused on neutralizing a single target, advances in biotherapeutic discovery now allow targeting multiple different antigens or pathways within a single molecule (231). With the advent of gene-editing technology, it has also become possible to engineer functionalities into human cells. In cancer, chimeric antigen receptor (CAR)-T cells have shown promise, and in autoimmunity, CAR–regulatory T cells (Tregs) are being pursued as a means to induce antigen-specific tolerance to autoantigens. As CRISPR technology advances rapidly, cells can be engineered to conduct multiple functions, and there is almost no theoretical limit to how complex such efforts can become. Accordingly, engineered cell therapies hold enormous promise.
3. Microbiota. Over the last decade, it has become increasingly clear that the microbes that inhabit us play vital roles in homeostasis and disease. A patient's microbiome may serve as an important prognostic or predictive disease biomarker, as has been suggested for treatment with checkpoint inhibitors in cancer immunotherapy (232). Multiple efforts to target microbiota in pursuit of a therapeutic effect are also currently underway, and these technologies hold particular promise in the area of mucosal diseases.
4. Understanding biological responses following intervention. With new technologies and increased sensitivities of technological platforms, our ability to interrogate human biology through DNA, epigenetics, single-cell transcriptomics, metabolomics, proteomics, exosomes, and imaging will provide significantly more insights into human biology and responses following therapeutic intervention. Analysis of differences between responders and nonresponders may reveal additional pathways for targeting.
5. Age of big data. As clinical, biomarker, and response data accumulate and are appropriately annotated, application of machine learning to these data sets may provide new therapeutic hypotheses to evaluate (233).

The many challenges to understanding human disease biology and developing novel therapeutics are balanced by these emerging novel technologies and insights. Their application will

undoubtedly enhance our understanding of human disease and increase our ability to provide clinical benefit for patients with as of yet unmet medical needs.

DISCLOSURE STATEMENT

Authors are all employees of Genentech, Inc. N.G., R.P, and J.R.A. are members of the Roche Group and hold stock and options in the Roche Group. A.C.C. is a member of the Roche Group and receives salary support and stock from Roche, Inc.

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