Program

Day I

Strategie per tollerizzare

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Intercept Pharmaceuticals

Topics

- Immune tolerance
- Autoimmune diseases
- Tolerance strategies

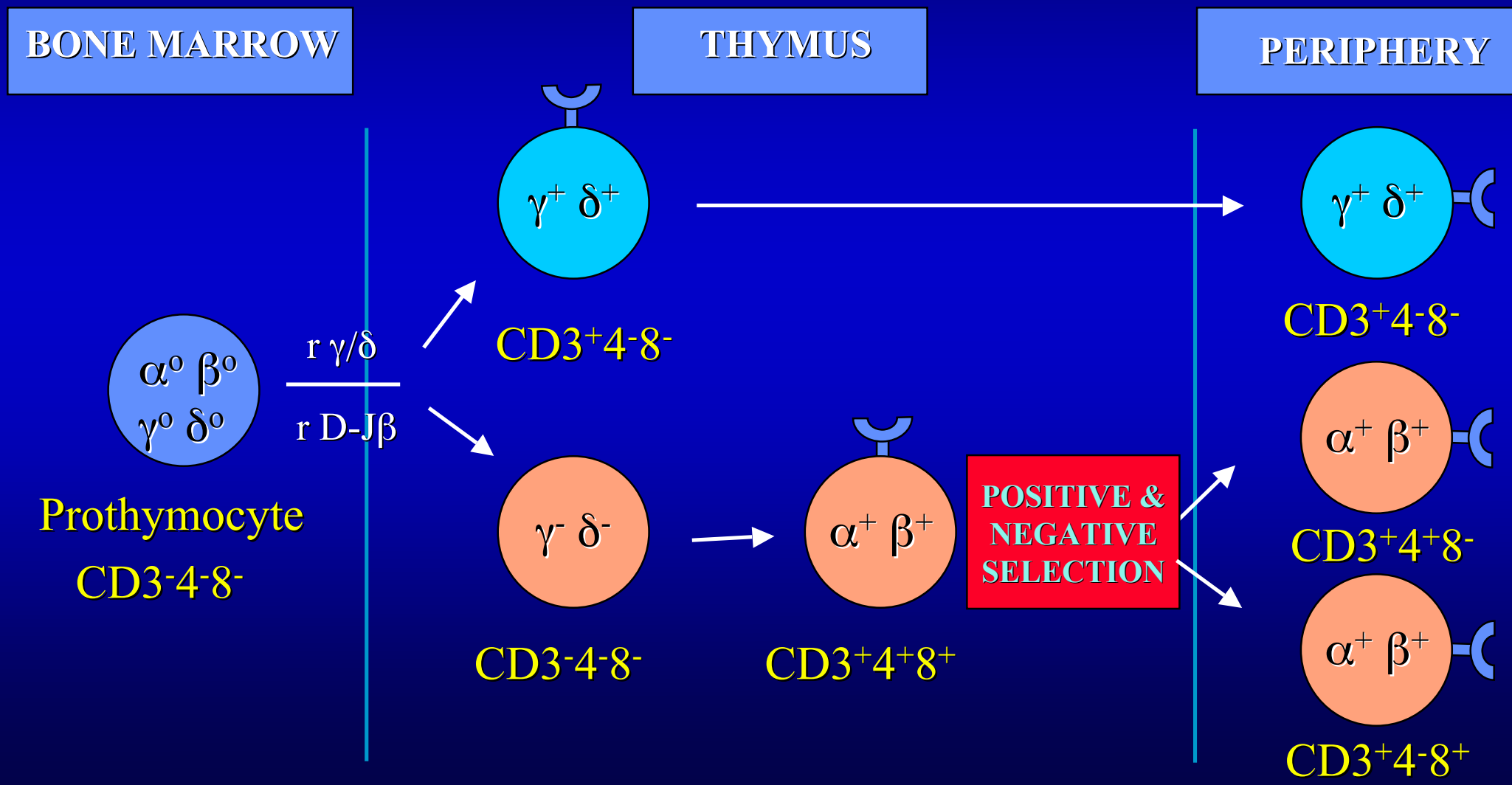
Immune tolerance: key concepts

- Immune tolerance is the inability of lymphocytes (T and B cells) to respond to self antigens
- Self tolerance can be divided into central and peripheral tolerance
- Tolerance is mediated by multiple mechanisms, but three are most important: deletion, anergy, suppression

Central vs. peripheral tolerance: key concepts

- In central tolerance, immature lymphocytes recognizing self antigens with high affinity in generative lymphoid organs (bone marrow for B cells and thymus for T cells) die by apoptosis
- in peripheral tolerance, mature self-reactive lymphocytes encounter self antigens in peripheral tissues and are killed or shut off by induction of anergy (functional unresponsiveness), deletion (apoptotic cell death), or suppression by regulatory T cells

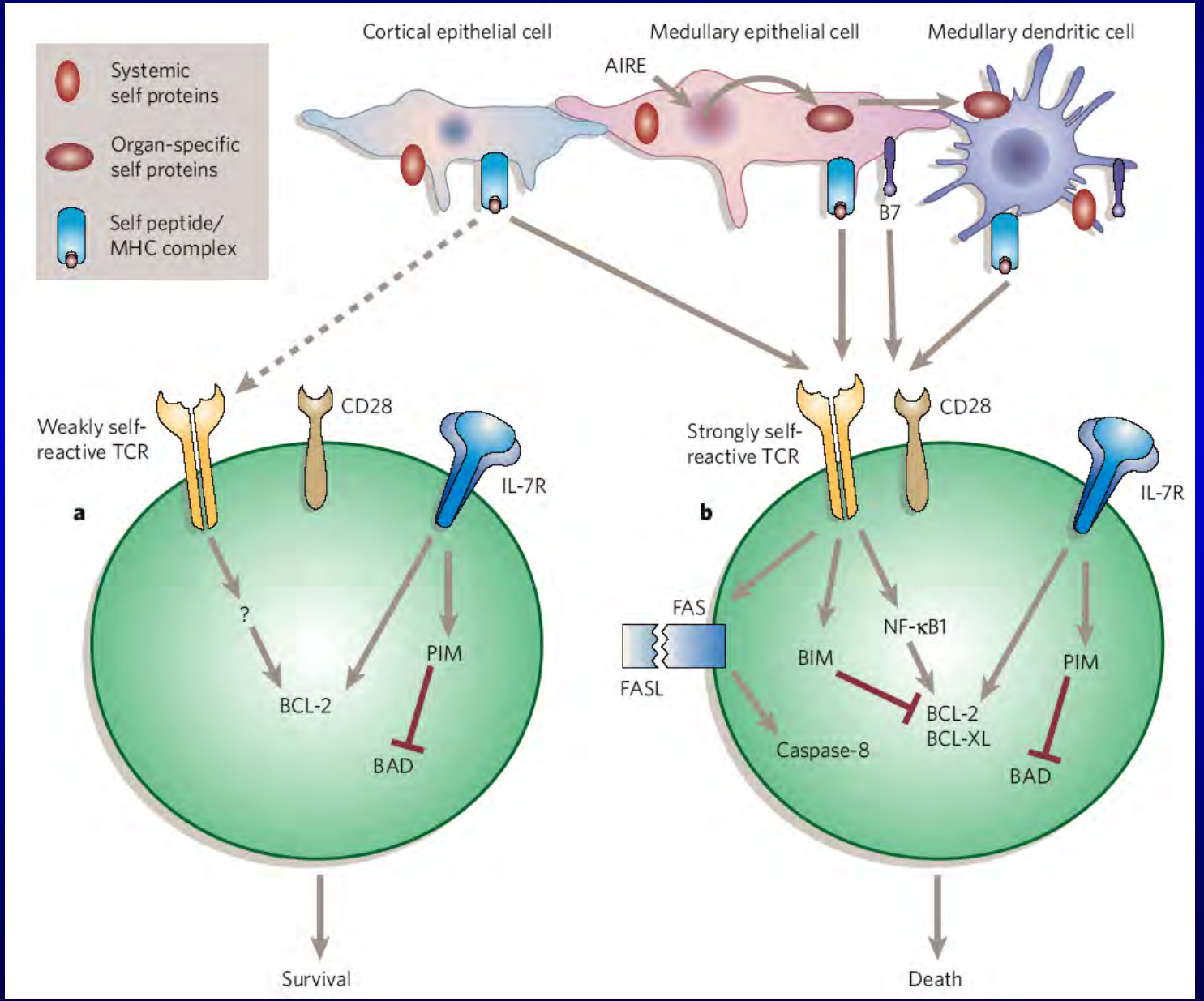
T cell development



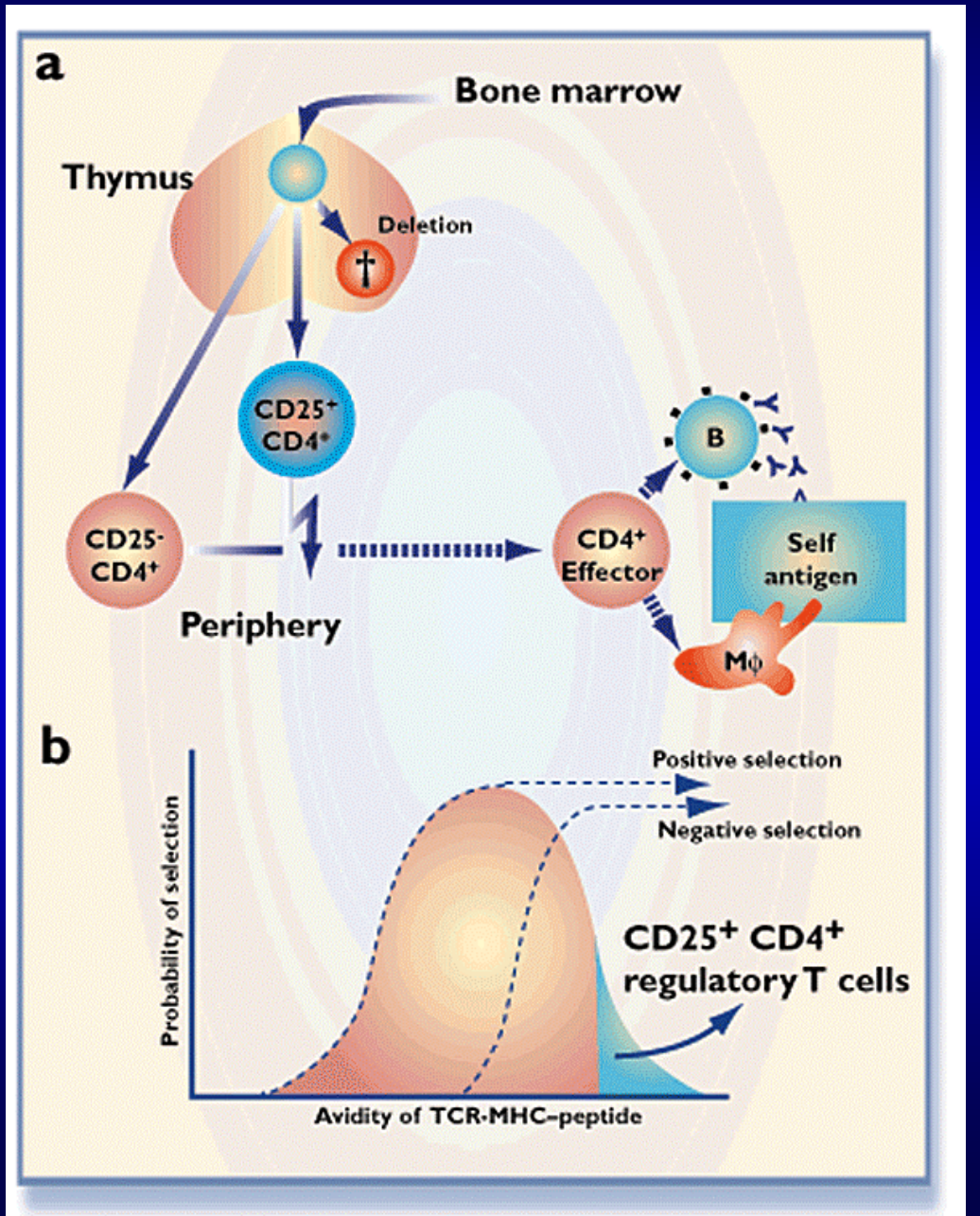
Critical events in intrathymic α/β T cell development

- Rearrangement of TCR α/β and expression of CD4 and CD8 co-receptors
- Positive selection of T cells with affinity for MHC-self peptide
- Down-regulation of CD4 or CD8
- Negative selection (apoptosis) by high-affinity binding to MHC-self peptide
- Export of single-positive cells

Pathways for deleting T cells with strongly and weakly self-reactive TCRs in the thymus



Thymic selection of T cells

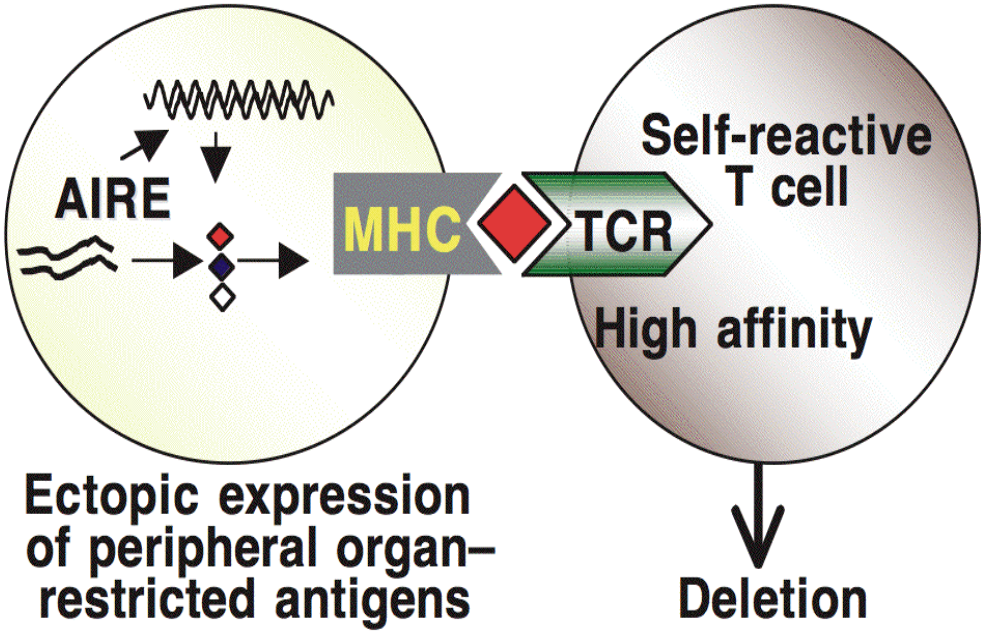


Sakaguchi, S. *Nature Immunology* **2**, 283 (2001)

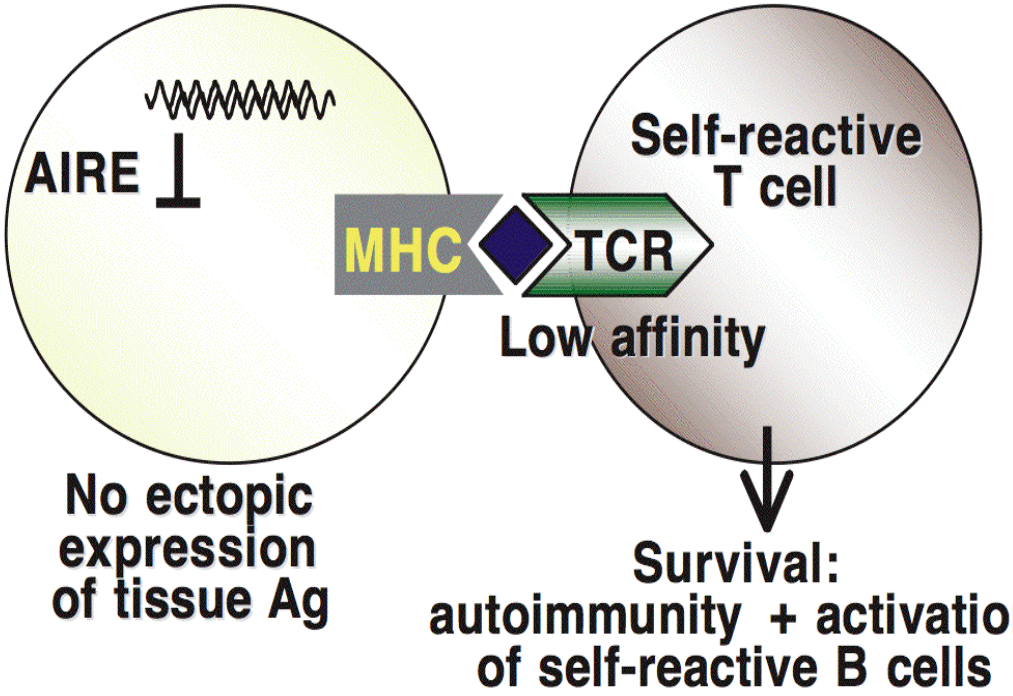
Genetic basis of autoimmune polyendocrinopathy candidiasis ectodermal dysplasia (APECED)

(Fischer, Nature Immunol 5:23-30, 2004)

1. Defective central tolerance



APECED SYNDROME (AIRE deficiency)

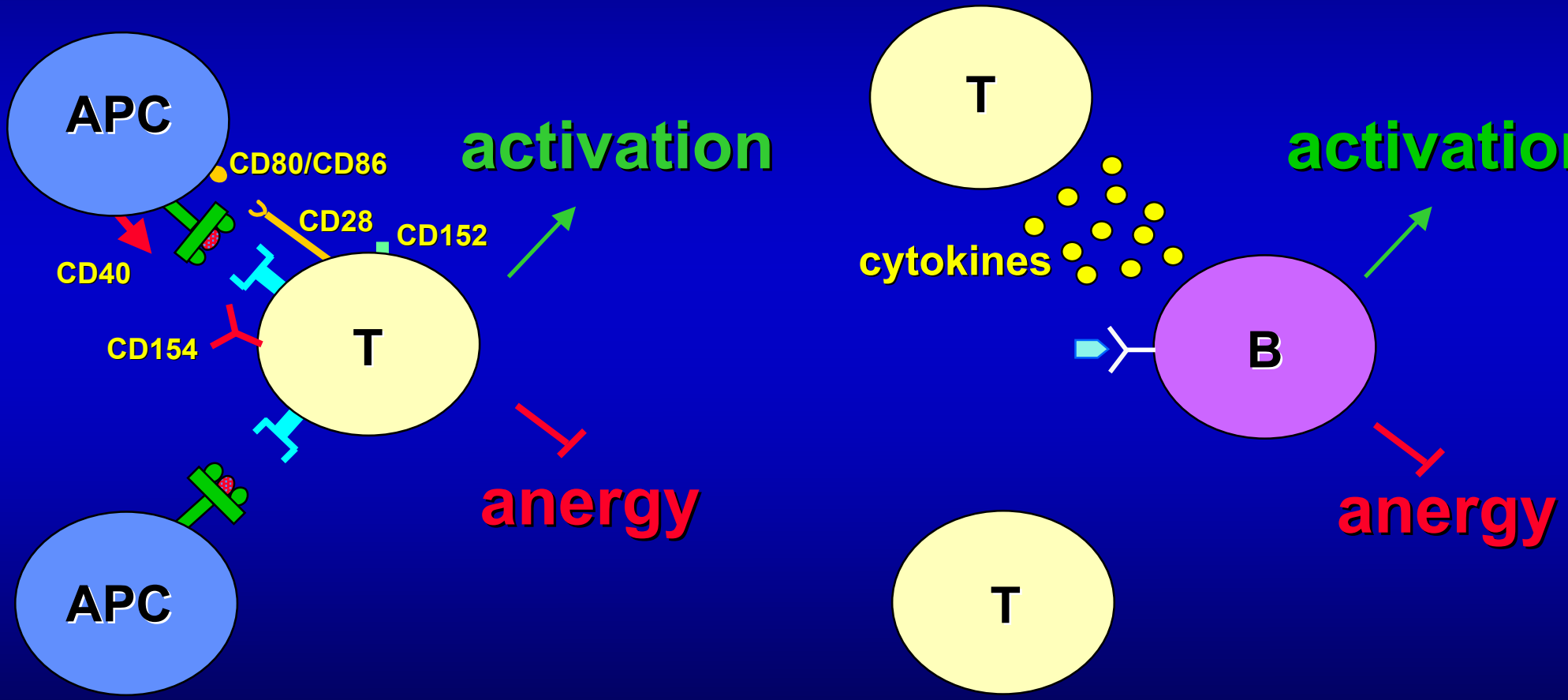


Mechanisms of peripheral tolerance

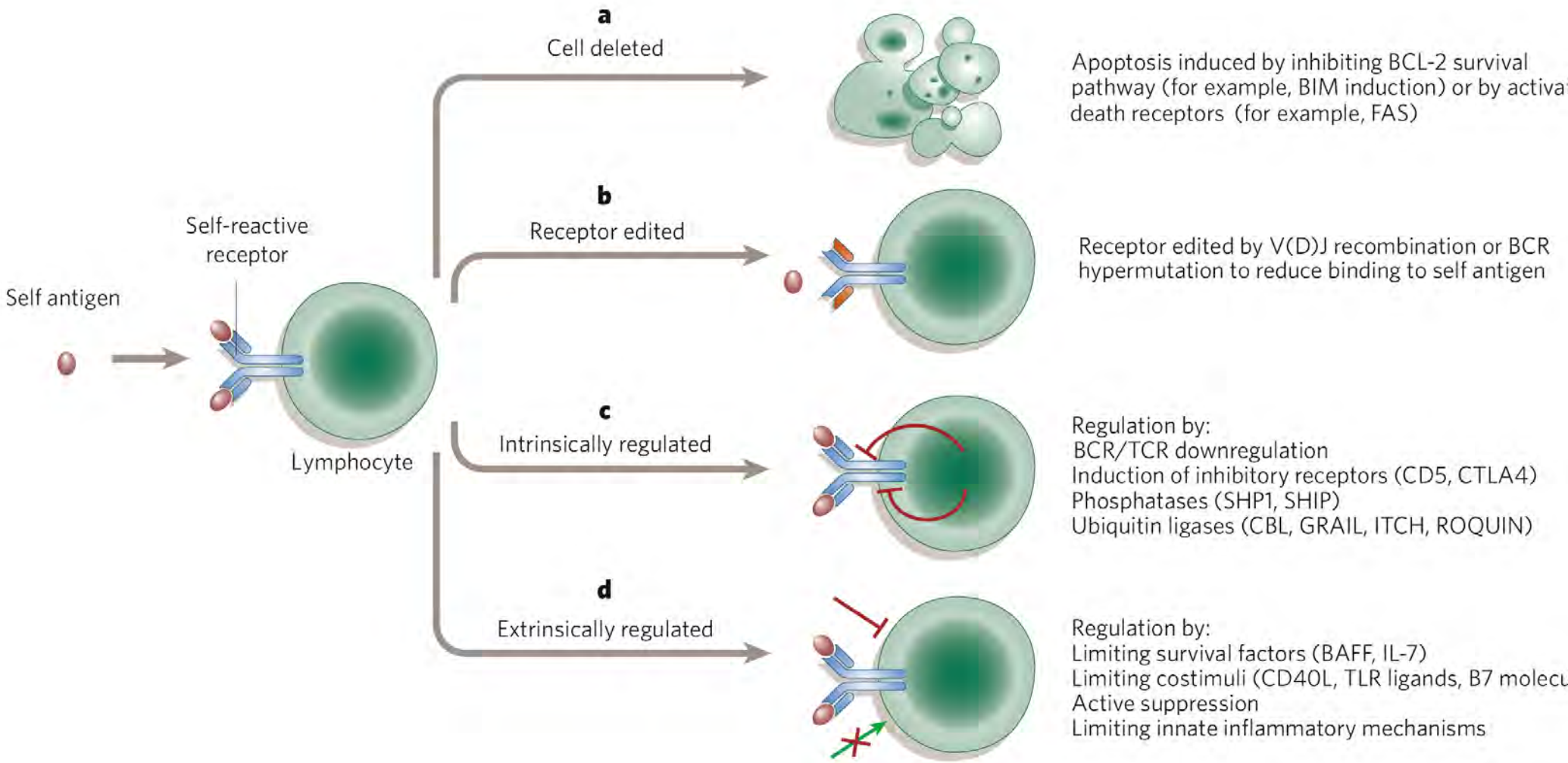
- Anergy
- Deletion
- Suppression by regulatory T cells
- Exhaustion
- Down-regulation of TCR, CD4/CD8
- Partial activation (Altered Peptide Ligands)

Activation vs. Anergy

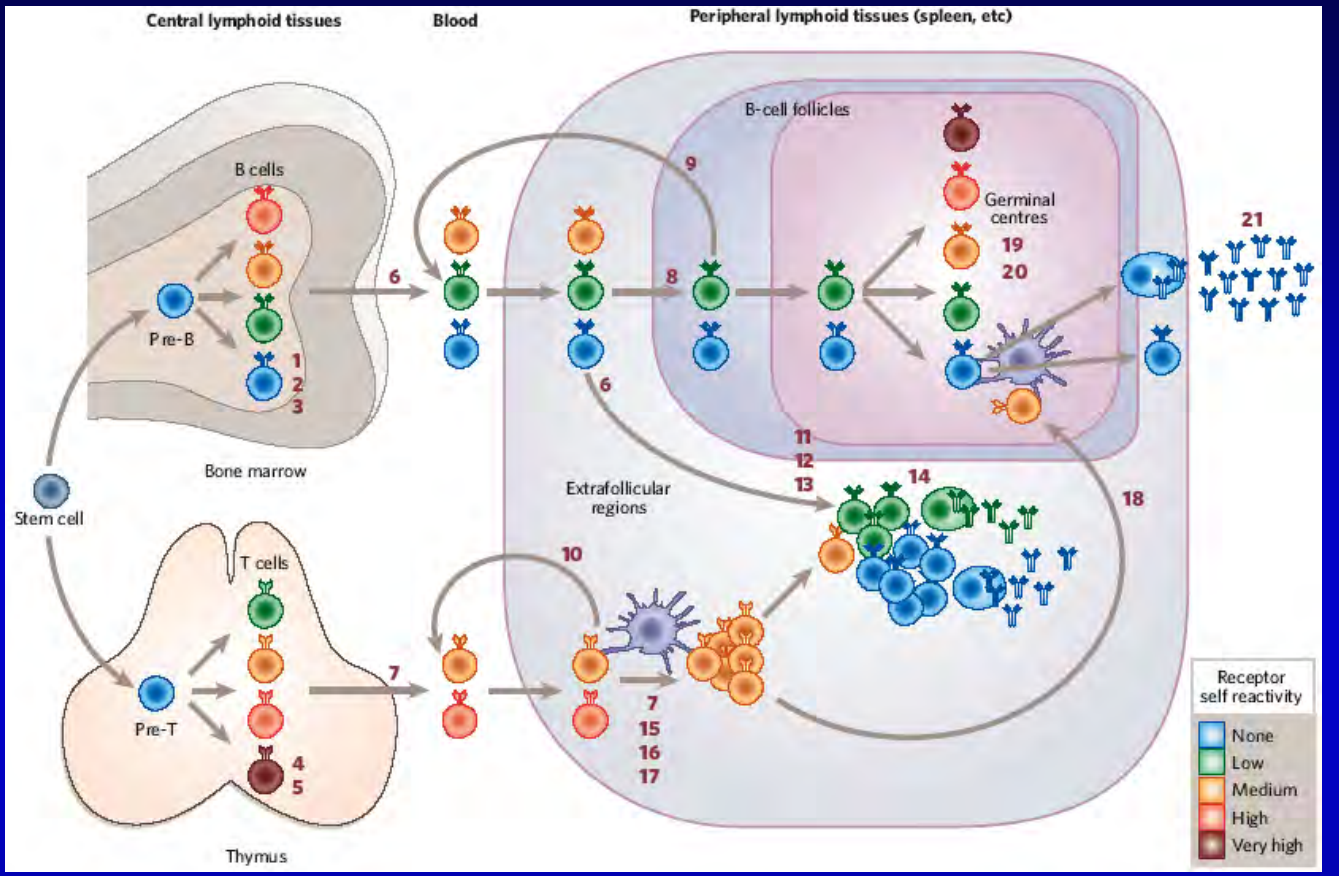
The two-signal theory of lymphocyte activation



Four cellular strategies regulate self-reactive receptors at different points during B- and T-cell differentiation



Cellular checkpoints regulating self-reactive receptors



BCR tolerance mechanisms in central lymphoid organs:
(1) Arrest of immature B-cell maturation
(2) BCR light chain editing by V(D)J recombination
(3) Death and deletion of immature B cells
TCR tolerance mechanisms in central lymphoid organs:
(4) TCR –chain editing by V(D)J recombination
(5) Death and deletion of semi-mature T cells
Intrinsic regulation of self-reactive receptors by anergy:
(6) BCR tuning/anergy
(7) TCR tuning/anergy
Extrinsic regulation of self-reactive receptors:
(8) Follicular exclusion of B cells
(9) B-cell competition for BAFF
(10) T-cell competition for IL-7

Extrinsic regulation of self-reactive receptors by limiting costimuli:
(11) Controls on availability of extrafollicular T-cell help
(12) Control of TLR ligands and signalling
(13) B-cell death induced by FASL from T cells
(14) BCR inhibition of plasma-cell differentiation
(15) Control of B7 ligands and other costimulatory molecules
(16) T-cell death induced by FASL
(17) T-cell suppression by T regulatory cells
Regulation of self-reactive receptors in follicles:
(18) Control of ICOS and follicular T helper cell differentiation
(19) BCR-induced death of germinal centre B cells
(20) Germinal-centre B-cell death from competition for follicular T helper cells
Tolerance of self-reactive receptors at the final effector phase:
(21) Control of autoantibody accumulation and inflammation in tissues

Etiopathogenesis of autoimmune diseases

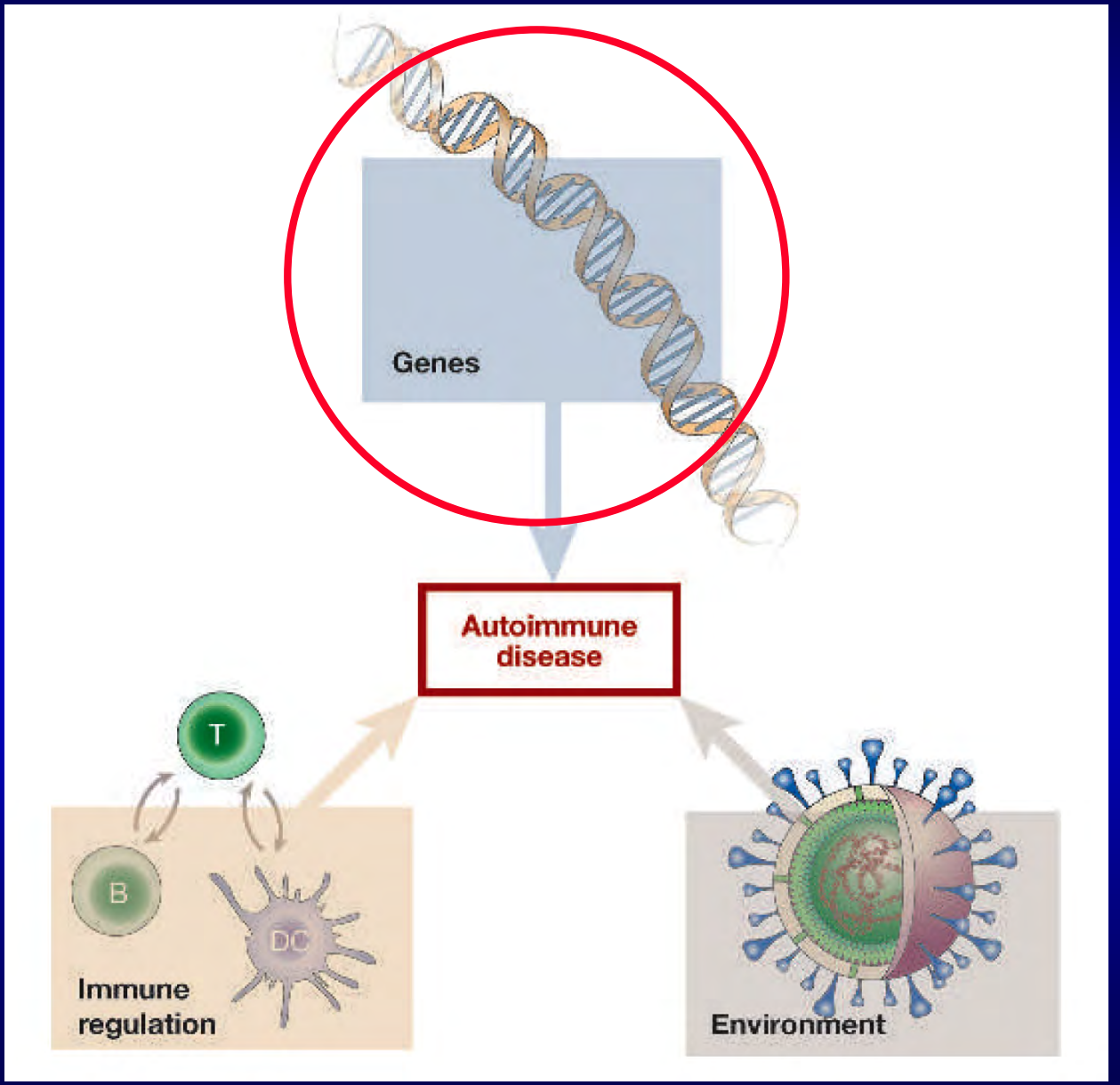
Loss of T cell tolerance to self caused by

- Bystander activation
- Molecular mimicry
- Decreased activity of regulatory/suppressor cells
- Defect in apoptosis
- Superantigens (polyclonal activation of T and B cells
- Increased expression of class II molecules
- Release of sequestered antigens

Characteristics of autoimmune diseases

- Chronic diseases, typically with a remitting-relapsing course
- Associated with particular HLA alleles
- Critical event: loss of tolerance to self leading to activation of autoreactive T cells
- Induced and/or maintained by autoreactive, pathogenic T and B cells
- Higher frequency in females

Requirements for autoimmune disease development

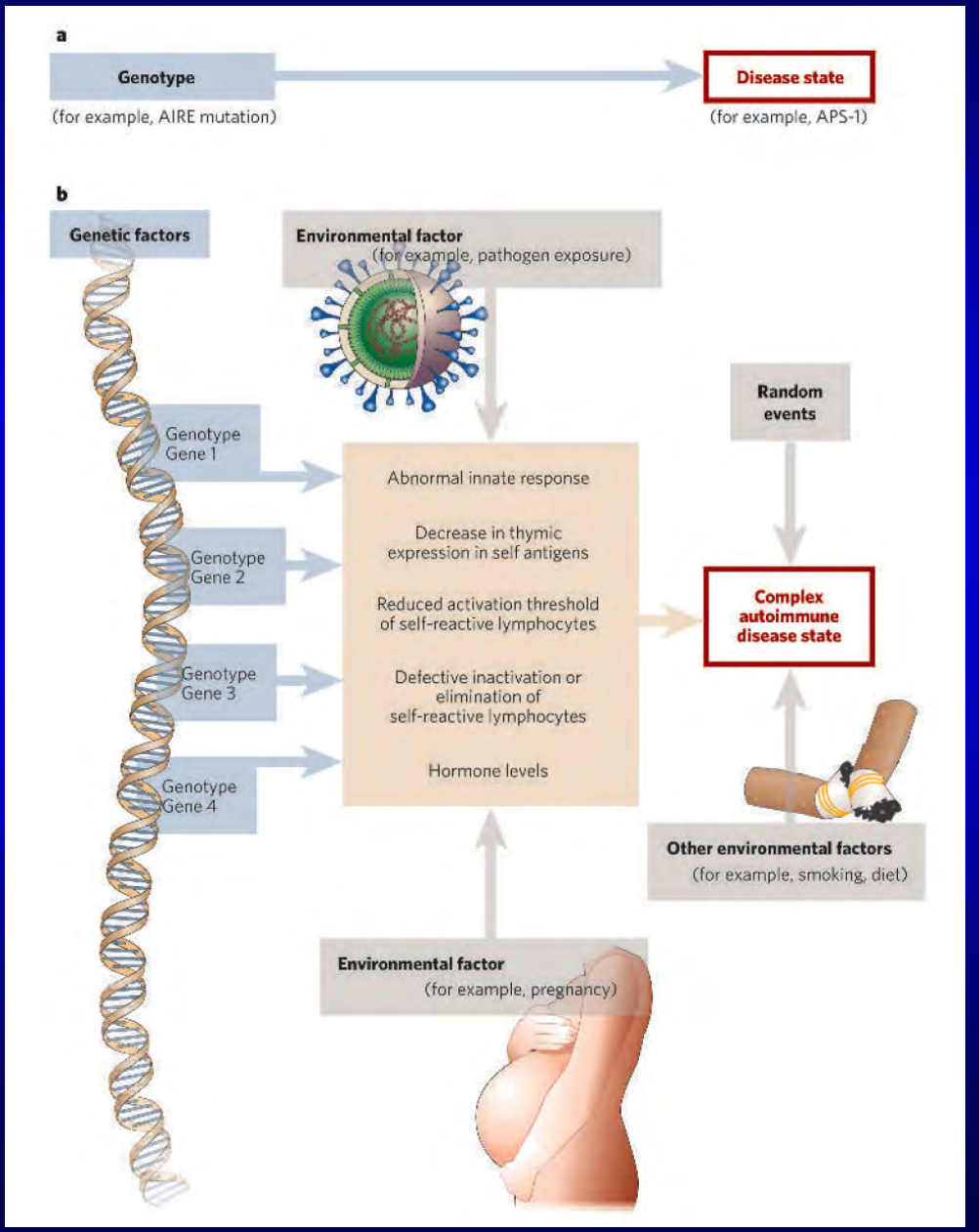


Single genetic traits associated with autoimmunity

Gene	Human disease	Mouse mutant or knockout	Mechanism of autoimmunity
AIRE	APS-1	Knockout	Decreased expression of self antigens in the thymus, resulting in defective negative selection of self-reactive T ce
CTLA4	Association with Graves' disease, type 1 diabetes and others	Knockout	Failure of T cell anergy and reduced activation threshold of self-reactive T
FOXP3	IPEX (scurfy)	Knockout and mutation	Decreased generation of $CD4^+$ $CD25^+$ regulatory T cells
FAS, FASL	ALPS	lpr/lpr; gld/gld mutants	Failure of apoptotic death of self-reactive B and T cells
C4 complement protein	Associated with SLE	Knockout	Defective clearance of immune compl and possible failure of B cell tolerance

AIRE: Autoimmune regulator
APS: Autoimmune polyendocrine syndrome
IPEX: immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome
ALPS: autoimmune lymphoproliferative syndrome

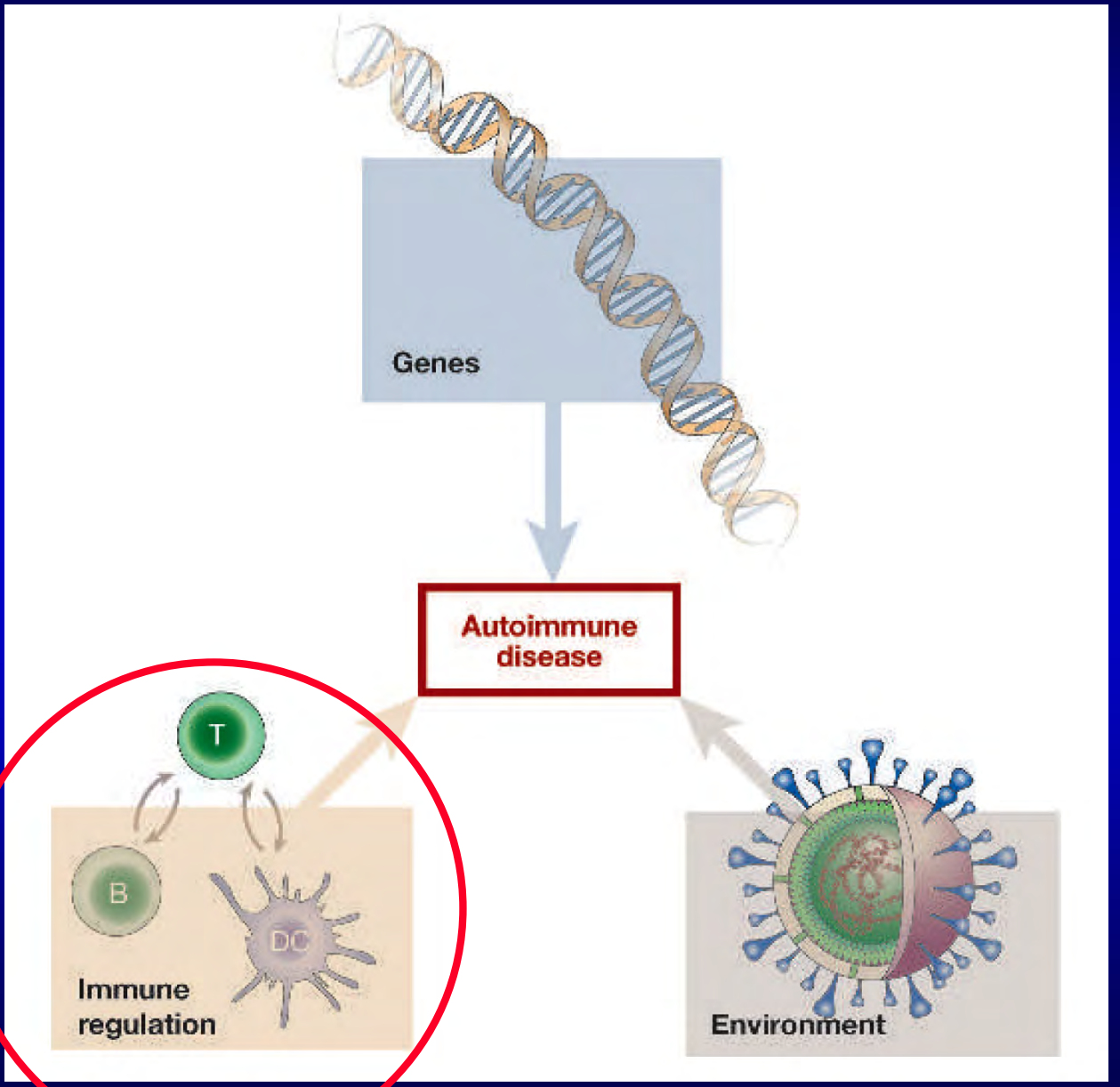
Single gene disorders vs. a model of autoimmune diseases caused by complex traits



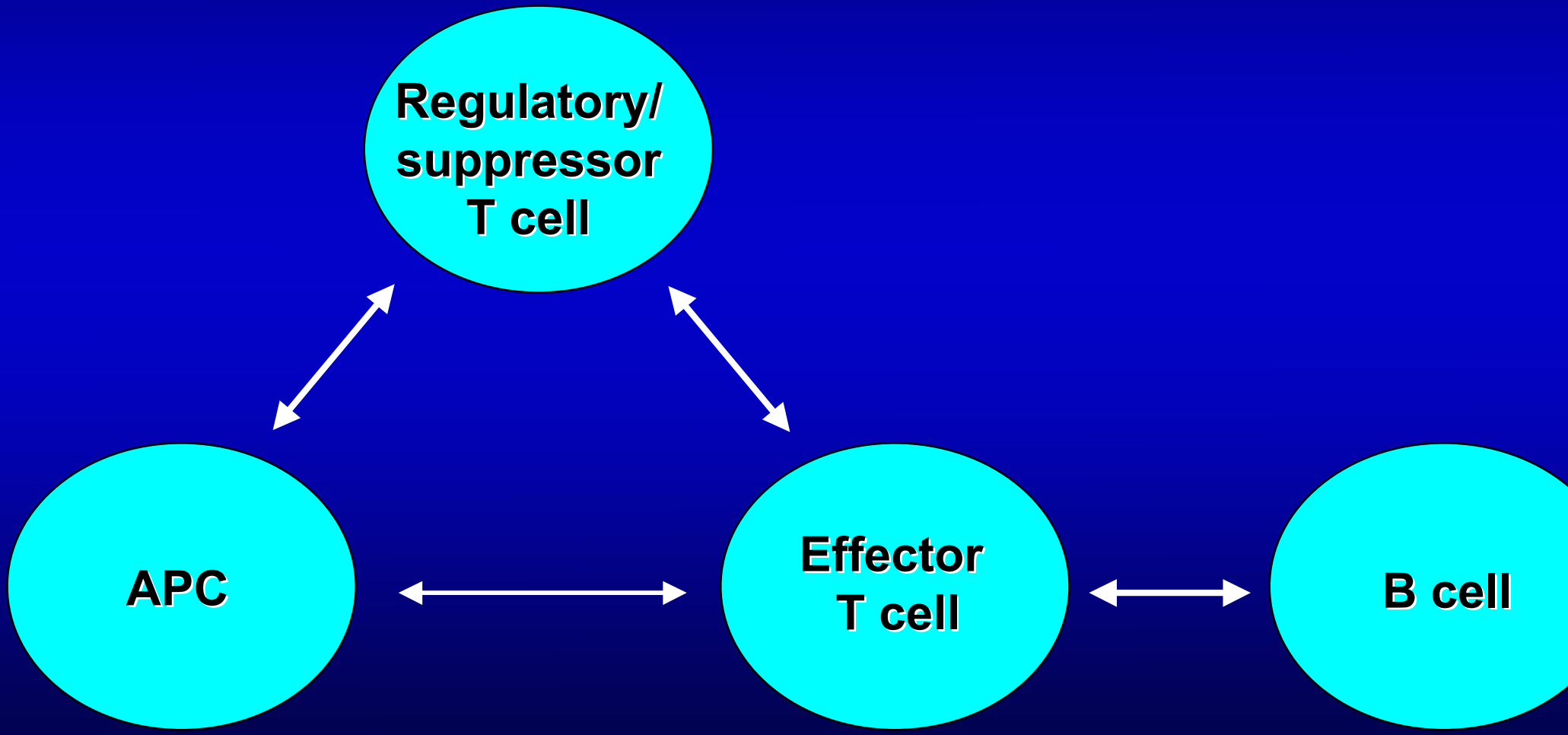
Immune genes associated with autoimmune diseases

- MHC class I and class II
- T Cell Receptor
- Immunoglobulin
- Cytokines

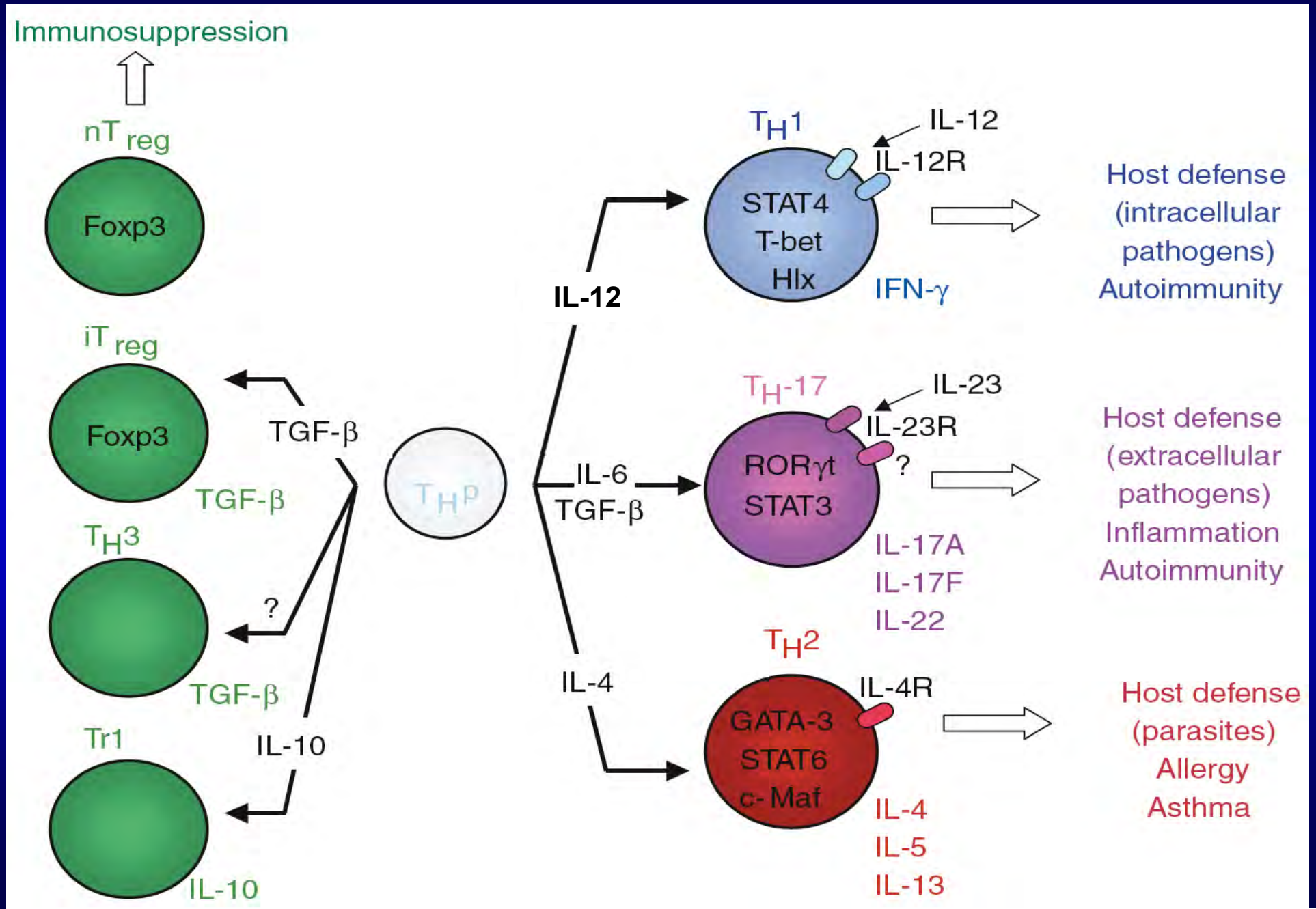
Requirements for autoimmune disease development



Key cells in autoimmune diseases

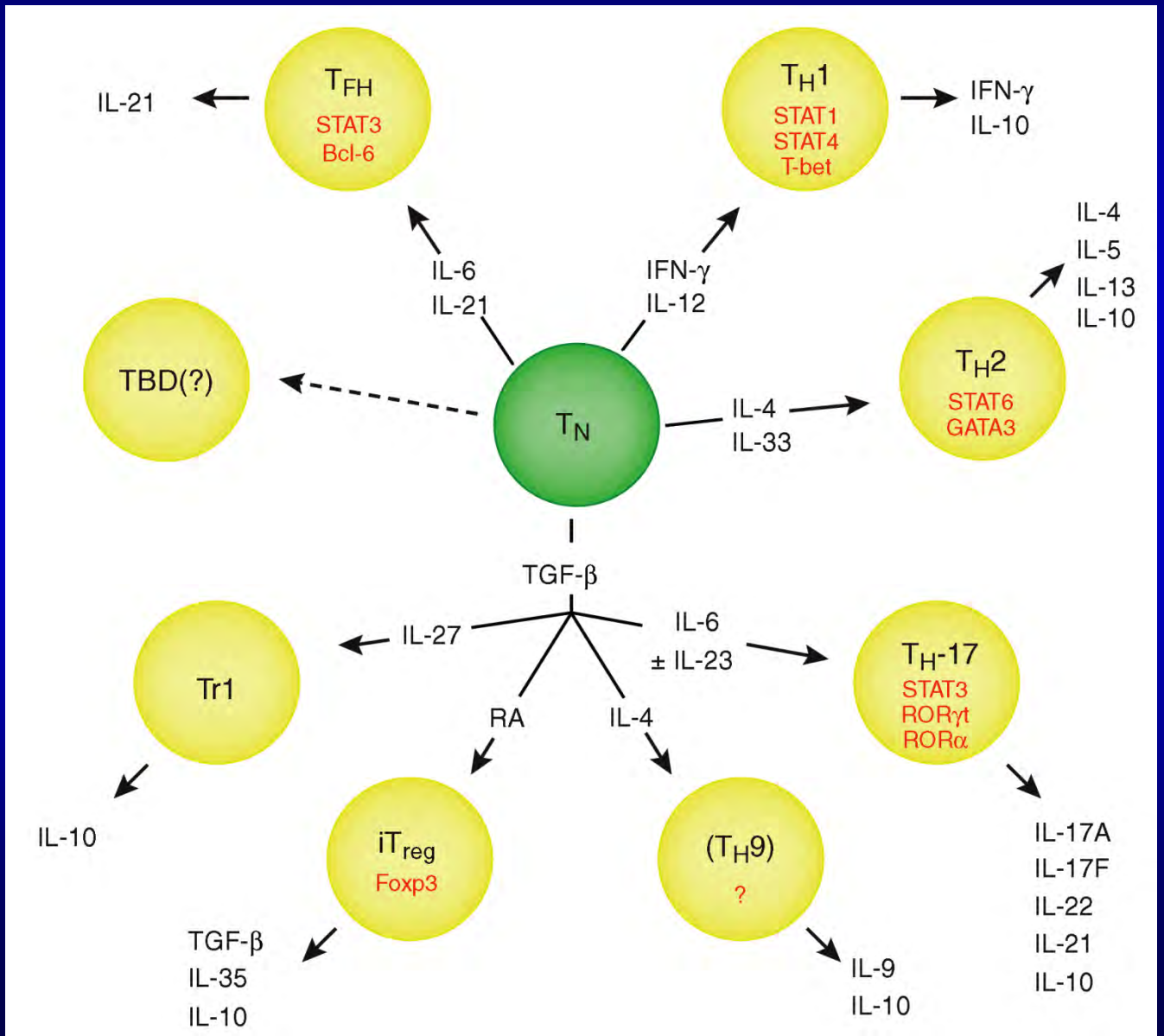


Differentiation of mouse CD4+ T cell lineages



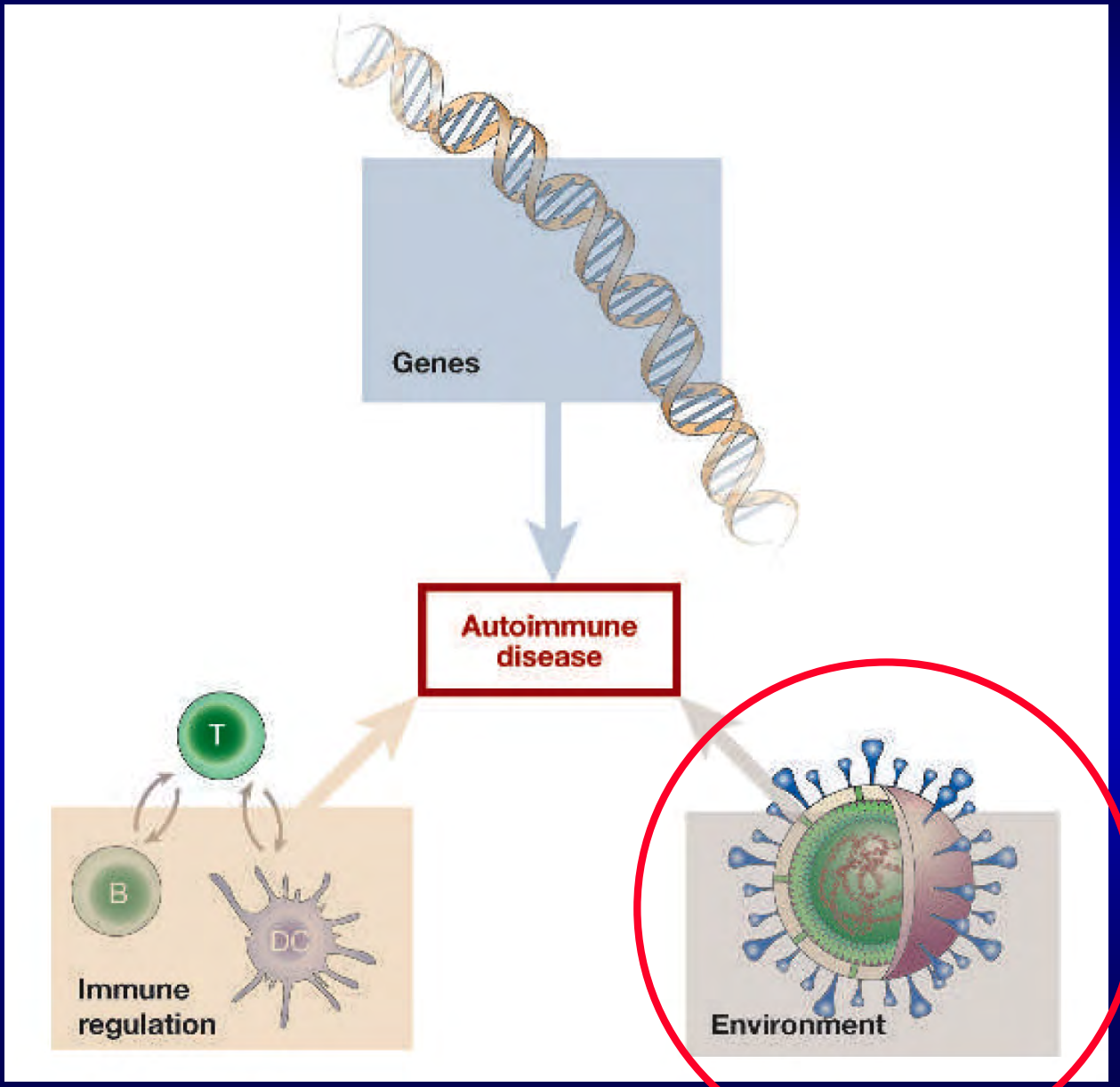
Bettelli et al., Nature Immunol. 8:345, 2007

Polarization of CD4+ T cell subsets

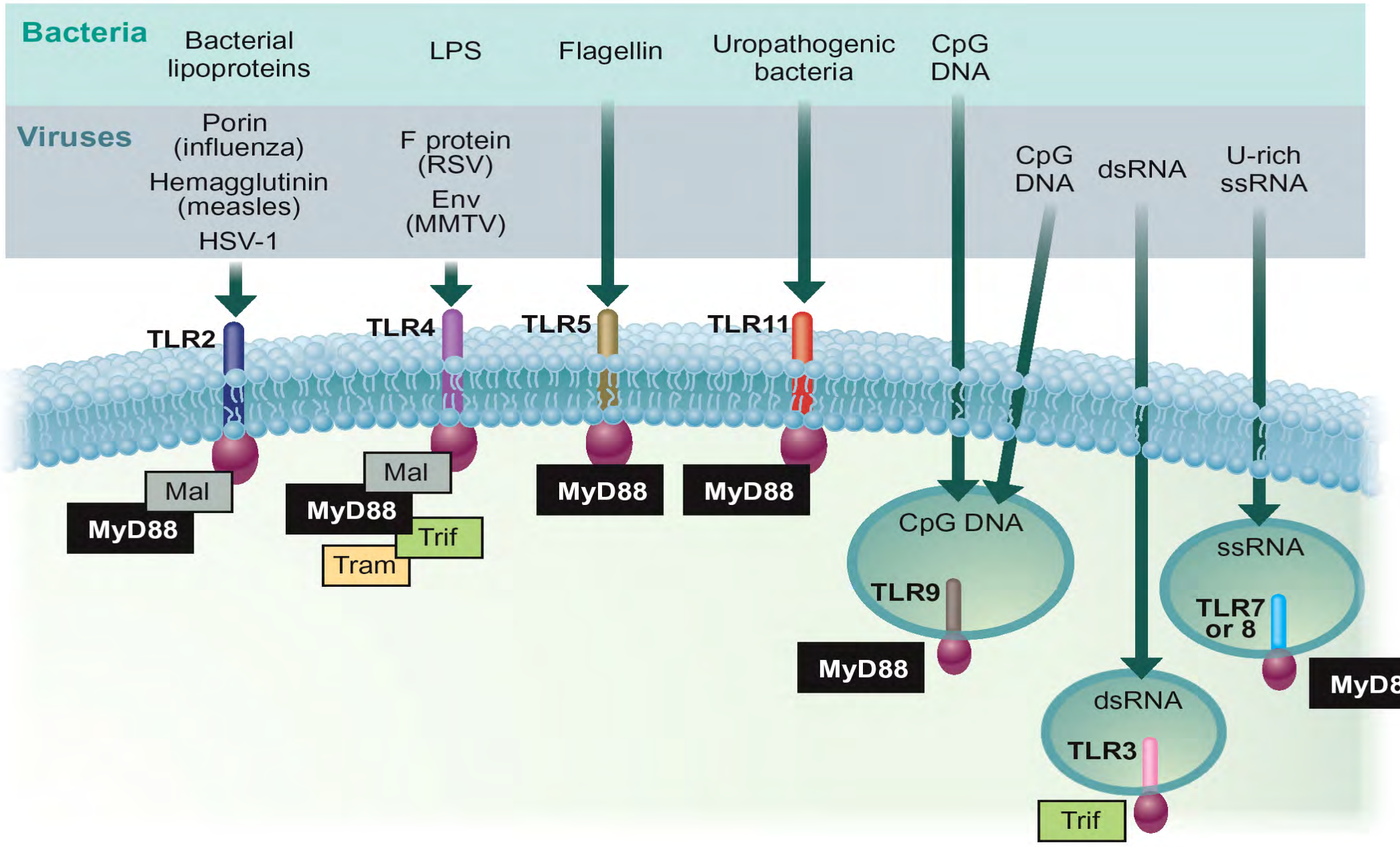


Palmer & Webe., Nature Immunol. 11:1, 2010

Requirements for autoimmune disease development



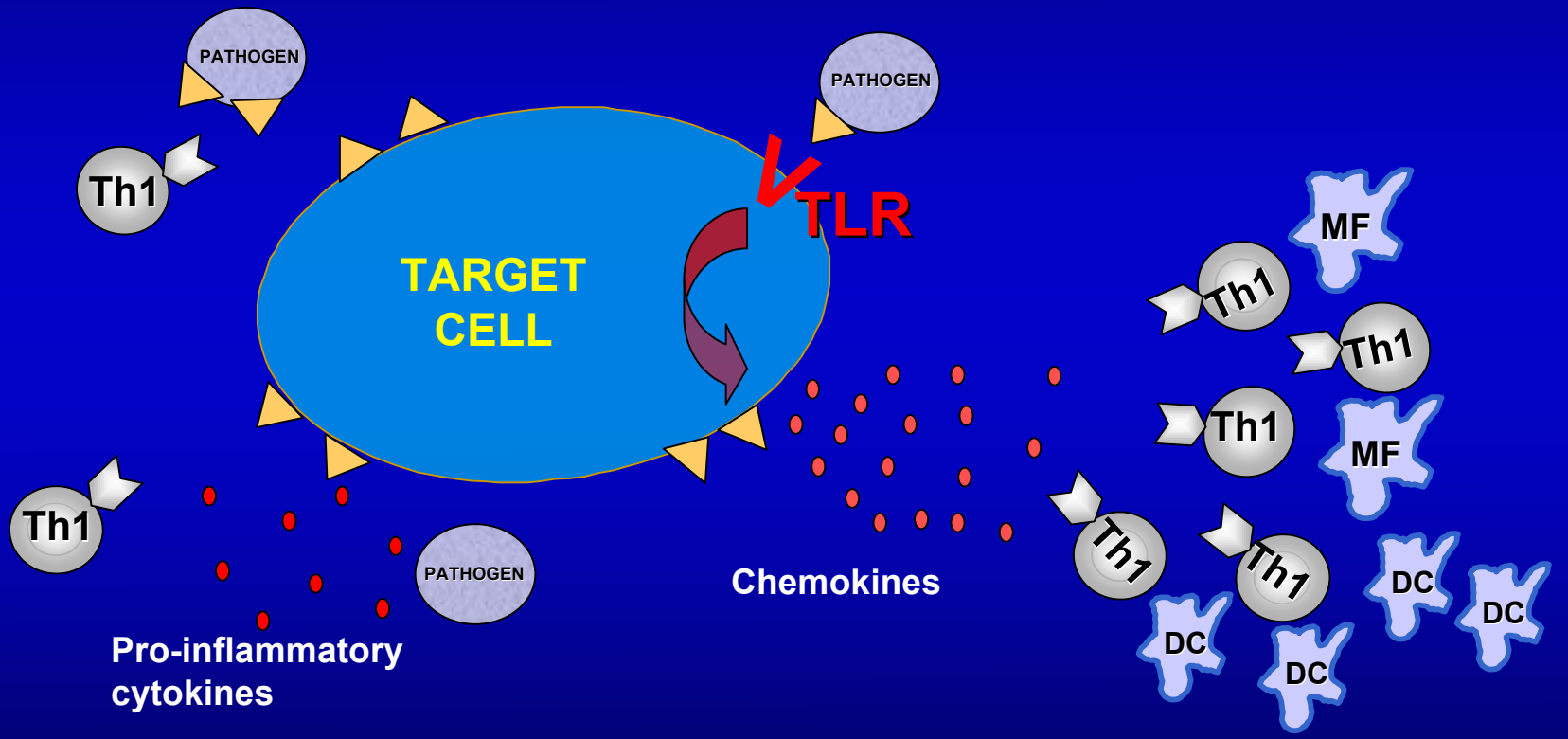
TOLL-LIKE RECEPTORS



PATHOGENS PROVOKE AUTOIMMUNE DISEASE ONSET: MECHANISTIC EXPLANATIONS

MOLECULAR MIMICRY

TLR LIGATION

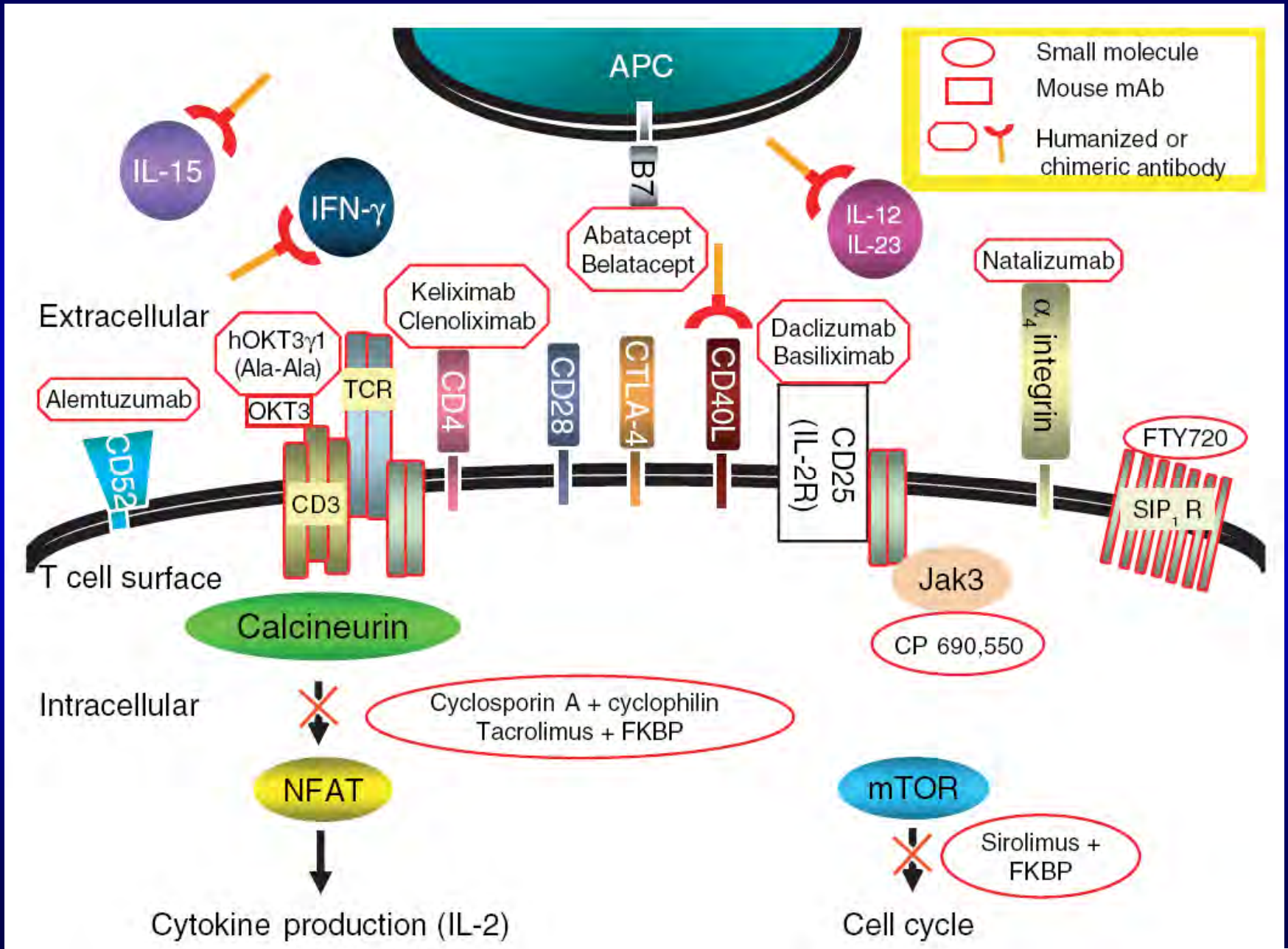


BYSTANDER ACTIVATION

Selective immunointervention in autoimmune diseases

- **Targeting pathogenic T cells**
  - MHC blockade, Antigen, APL, anti-CD3
- **Targeting costimulatory molecules**
  - Co-receptors (CD4), Costimulation blockade, Coinhibition upregulation
- **Cytokine-based immunointervention**
  - Inhibition of inflammatory cytokines (IL-1, IL-2, IL-6, IFN-γ, IL-12, TNF-α, IL-1
  - Administration of anti-inflammatory cytokines (IL-4, IL-10, IFN-α/β, TGF-β)
- **Induction of regulatory T cells**
  - T cell/TCR peptide vaccination, APC manipulation, cytokines
- **Targeting leukocyte trafficking**
  - Adhesion molecules, chemokines, chemokine receptors

T cell–directed therapy



T cell–directed therapy: Small molecules

Small molecules	Other names	Mechanism of action	Main side effects
Cyclosporin	Neoral; Sandimmune; SangCya	Binds cyclophilin to reduce production of cytokines (IL-2)	Nephrotoxicity, hypertension, hypercholesterolemia
Tacrolimus	Prograf; FK506; Protopic (topical)	Binds FKBP to reduce production of cytokines	Nephrotoxicity, diabetes, tremor, hypertension
Sirolimus	Rapamycin; Rapamune	Binds FKBP and reduces mTOR to inhibit response to cytokines (IL-2)	Hyperlipidemia, peripheral edema, oral ulcers, myelosuppression
FTY720	Fingolimod	Inhibits lymphocyte trafficking by $S1P_1$ signaling	Bradycardia, lymphopenia, constipation
CP 690-550		Inhibits Jak3 signaling	Anemia

T cell–directed therapy: biologicals

Anti-thymocyte globulin	ATGAM (horse); Thymoglobulin (rabbit)	Polyclonal antibody to T cells	Fever and chills, hypotension, thrombocytopenia
OKT3	Muromonab-CD3	mAb to CD3	Pulmonary edema, cytokine storm, thrombocytopenia
hOKT3g1 (Ala-Ala)		mAb to CD3	Fever, rash
Alemtuzumab	Campath 1H	mAb to CD52	Fever and chills, hypotension, thrombocytopenia
Keliximab, clenoliximab	IDEC-CE9.1; IDEC-151; SB-217969	Chimeric human-primate mAb to CD4	
Abatacept	CTLA-4–Ig; Orencia	Blocks CD28-B7 costimulation	Headache, infection
Belatacept	LEA29Y	Modified CTLA-4–Ig blocking CD28-B7 costimulation	
Ipilimumab	MDX-010	Anti-CTLA-4	Autoimmunity
Anti-CD28	TGN1412	'Super-agonistic' mAb to CD28	Cytokine storm, organ failure
Anti-p40	ABT-874/J695	mAb to IL-12 and IL-23	Injection site reaction, arthralgia, fever
Fontolizumab	HuZAF	Humanized mAb to IFN-γ	Abdominal pain, vomiting
Daclizumab	Zenepax	Humanized mAb to IL-2 receptor (CD25)	Fever and chills
Basiliximab	Simulect	Chimeric mAb to IL-2 receptor (CD25)	Fever and chills
Natalizumab	Tysabri; Antegren	mAb to integrin α_4, blocks cell adhesion	Influenza-like symptoms

Tolerogenic agents

- Autoantigens
- Biologicals
- Small molecules
- Treg cell therapy

Specific antigen targets in organ-specific autoimmune diseases

Disease	Antigen
Myasthenia gravis	Acetylcholine receptor
Multiple sclerosis	MBP, MOG, PLP
Pemphigus vulgaris	Desmoglein-3
Rheumatoid arthritis	Filaggrin
Hashimoto's thyroiditis	Thyroid peroxidase, thyroglobulin
Graves' disease	Thyrotropin receptor
Type 1 diabetes	GAD, insulin, HSP 60

GAD Treatment and Insulin Secretion in Recent-Onset Type 1 Diabetes

N ENGL J MED 359;18 WWW.NEJM.ORG OCTOBER 30, 2008

BACKGROUND

The 65-kD isoform of glutamic acid decarboxylase (GAD) is a major autoantigen patients with type 1 diabetes mellitus. This trial assessed the ability of alum-form lated GAD (GAD-alum) to reverse recent-onset type 1 diabetes in patients 10 to years of age.

CONCLUSIONS

GAD-alum may contribute to the preservation of residual insulin secretion in p tients with recent-onset type 1 diabetes, although it did not change the insulin quirement. (ClinicalTrials.gov number, NCT00435981.)

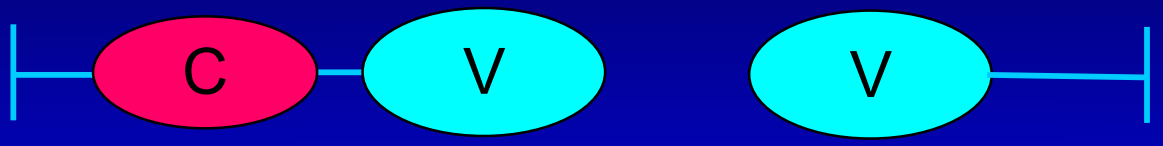
Potential targets for therapeutic agents promoting tolerance

Mechanism	Target
Co-stimulation blockade	CD28/B7; CD154/CD40; LIGHT/HVEM; ICOS/ICOSL; CD134/CD134L
Negative co-regulator activation	CD152 (CTLA-4); PD-1
Ts cell induction	VDR; IL-10R
DC maturation/function	NF-κB; VDR; ILT3/ILT4

DC membrane costimulatory molecules

- B7 family members: ligands for CD28, CD152 (CTLA-4), ICOS, PD-1
- Membrane-bound TNF and TNF receptor family members
- CD54 (ICAM-1), CD102 (ICAM-2), CD50 (ICAM-3): ligands for CD11a/CD18 (LFA-1)
- CD58 (LFA-3): ligand for CD2
- T1/ST2L: ligand for T1/ST2

B7 family ligands and receptors



Expression	Ligands	Receptors	Expression	Functio
DC, B, M (induc)	CD80 (B7.1)	CD28	T (const)	↑
DC, B, M (const)	CD86 (B7.2)	CD152 (CTLA-4)	T (activated)	↓
DC, B, M (const)	ICOS-L (B7h)	ICOS	T (activated)	↑
DC, B, M, T (induc)	PD-L1 (B7-H1)	PD-1	T, B. M (activated)	↓
DC, B, M (induc)	PD-L2 (B7-H1)			
DC, T, M (induc)	B7-H3	?	?	↑

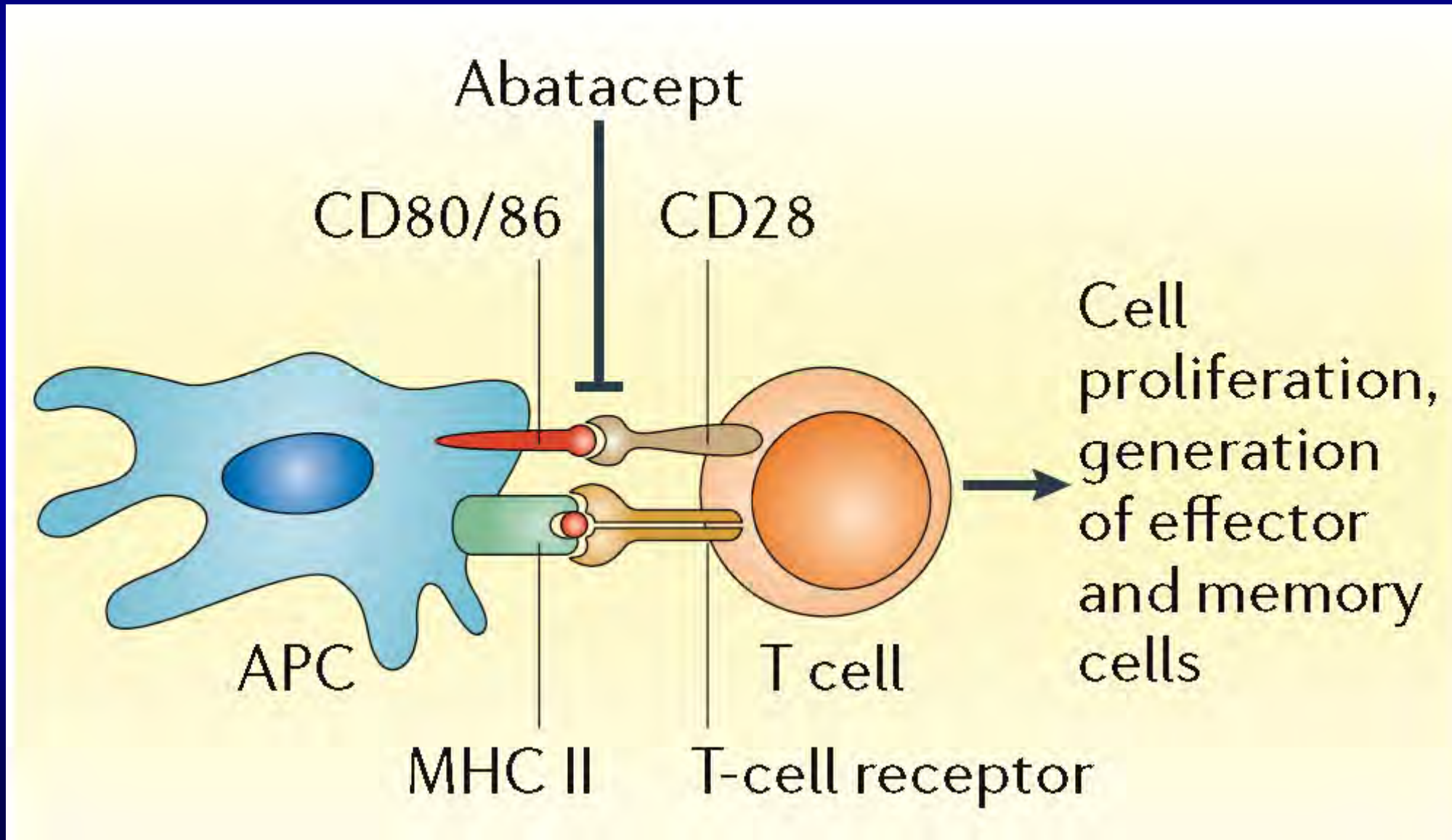
Biological immunomodulatory agents clinically effective in autoimmune diseases

Agent	Target
Infliximab, etanercept, onercept	TNF-α
IFN-β	IFN-β R
Anakinra	IL-1 Ra
Tocilizumab	IL-6 Ra
Alefacept	CD2
hOKT3g1 ala-ala	CD3
OKTcdr4a	CD4
Efalizumab	CD11a
Rituximab	CD20
Epratuzumab	CD22
Daclizumab, basiliximab	CD25 (IL-2Rα)
Alemtuzumab	CD52
Natalizumab	VLA-4
Abatacept	CD28

CTLA4-Ig (Abatacept) in the treatment of autoimmune diseases

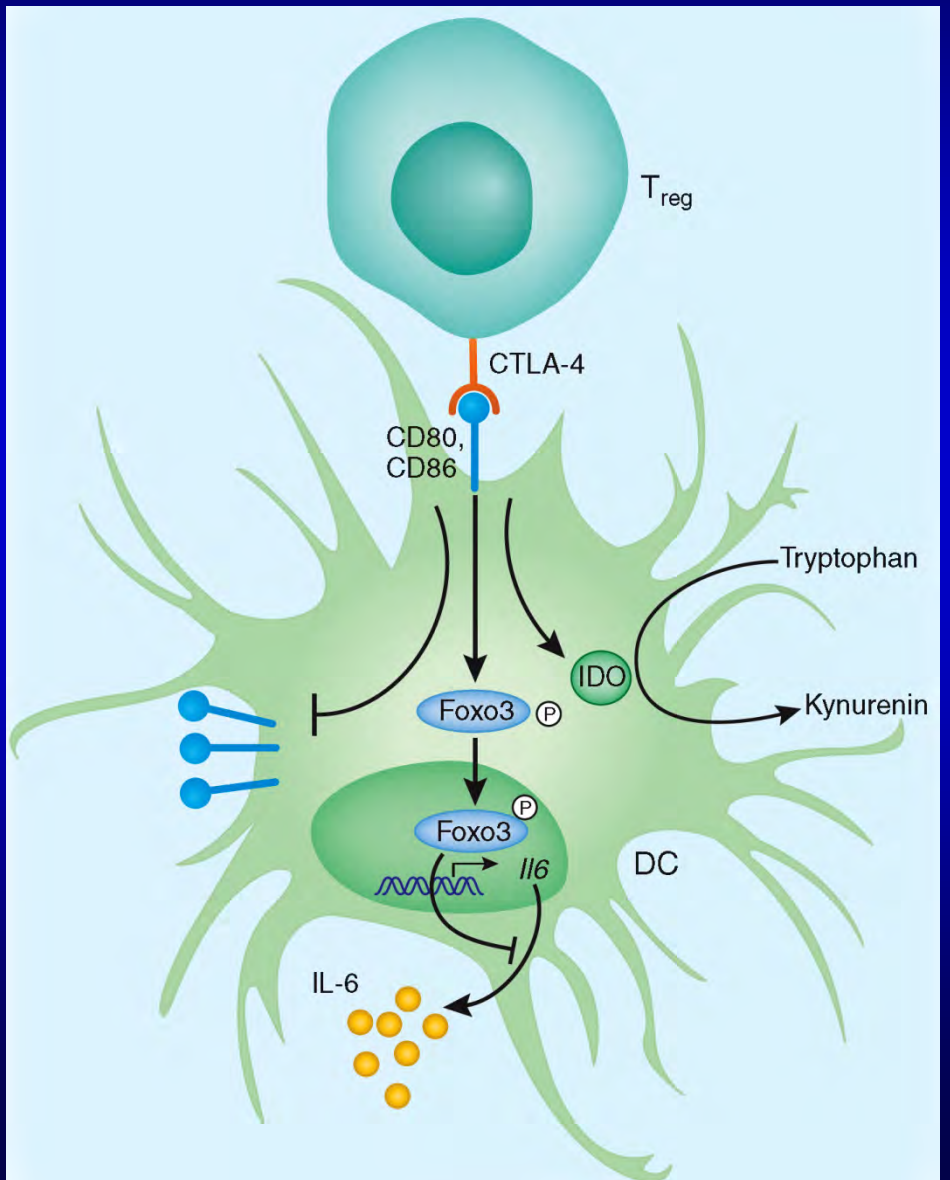
- **Rheumatoid Arthritis (FDA approved)**
- **JRA (FDA approved)**
- **Psoriasis (phase II)**
- **LES (phase II)**
- **Multiple Sclerosis (phase I/II)**

CTLA4-Ig (Abatacept) blocks T-cell costimulation



CTLA-4 may be a core mechanism through which Treg cells control APC function

Wng & Sakaguch., Nature Immunol. 11:7, 2010



Genovese MC, Becker JC, Schiff M, et al. Abatacept for rheumatoid arthritis refractory to tumor necrosis factor α inhibition. *N Engl J Med*. 2005;353:1114–1123.

TABLE 3 Selected endpoints

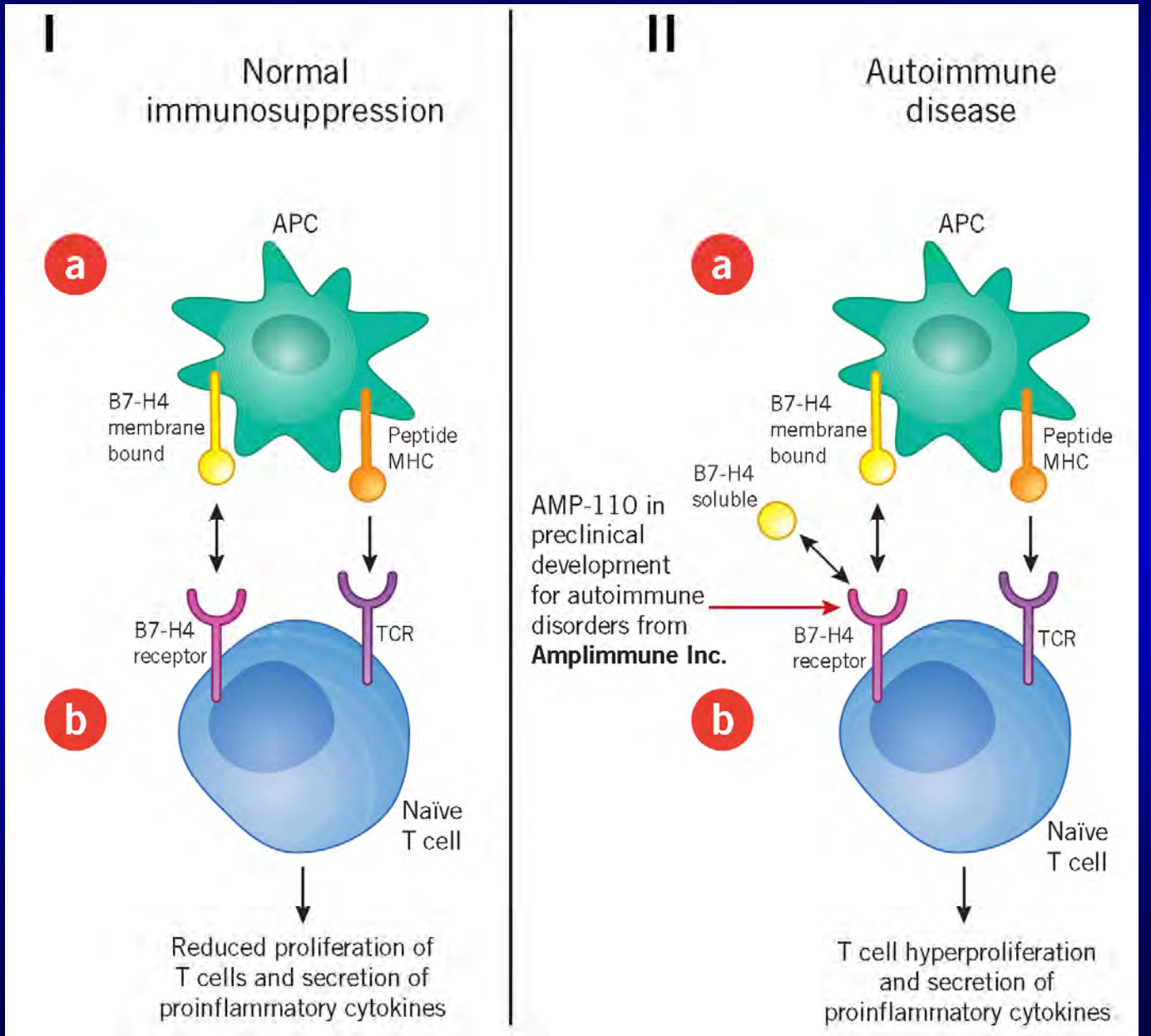
	Abatacept	Placebo	*P* value
ACR 20*	50.4%	19.5%	<.001
ACR 50	20.3%	3.8%	<.001
ACR 70	10.2%	1.5%	.003
≥0.3-point improvement in HAQ disability index*	47.3%	23.3%	<.001
SF-36 physical-component summary score (mean change from baseline)†	7	1	<.001
SF-36 mental-component summary score (mean change from baseline)†	5	2	<.01

*Primary endpoint.

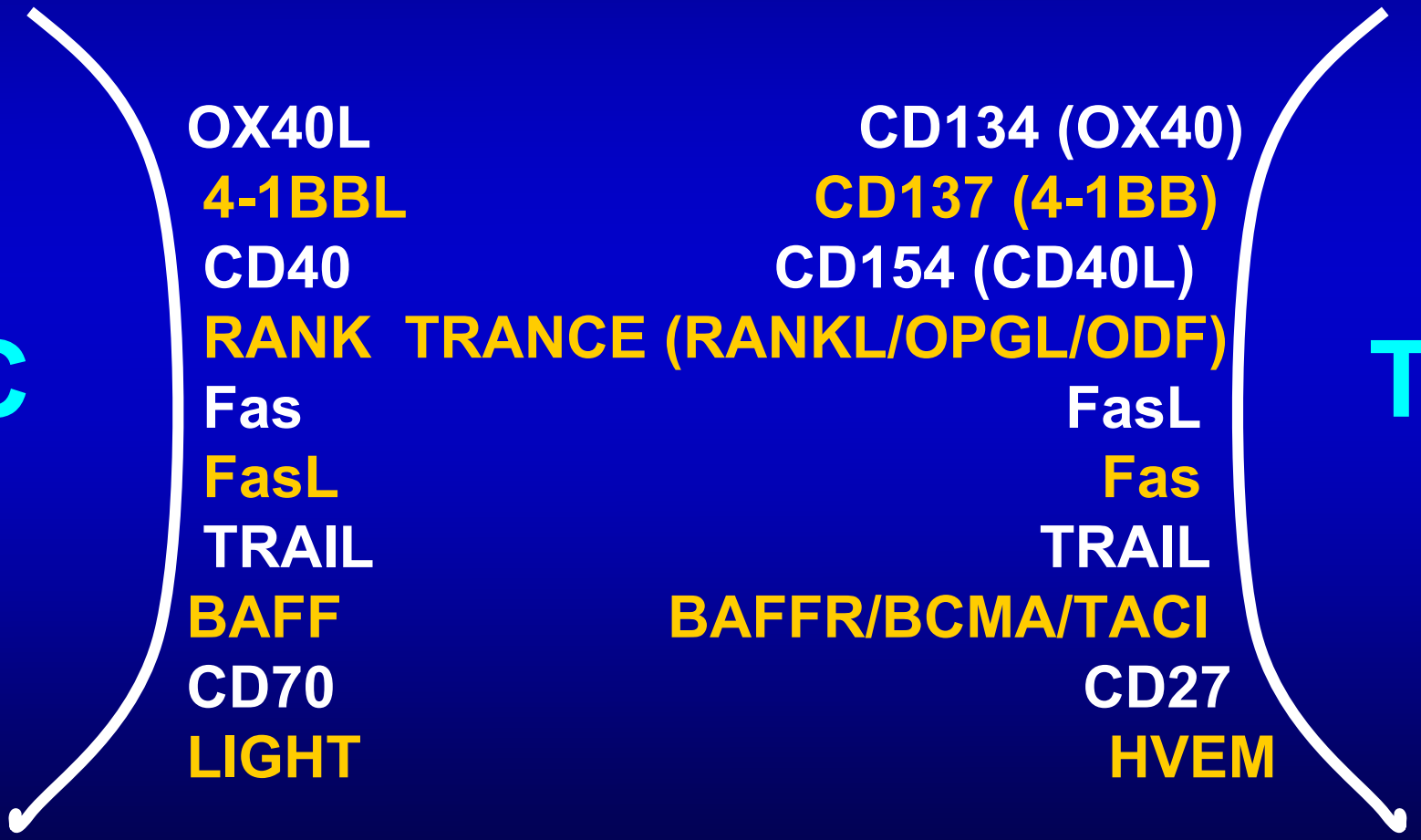
†Scores range from 0–100, with a higher score indicating better quality of life. An improvement of 3 points was considered clinically meaningful.

ADAPTED FROM GENOVESE 2005

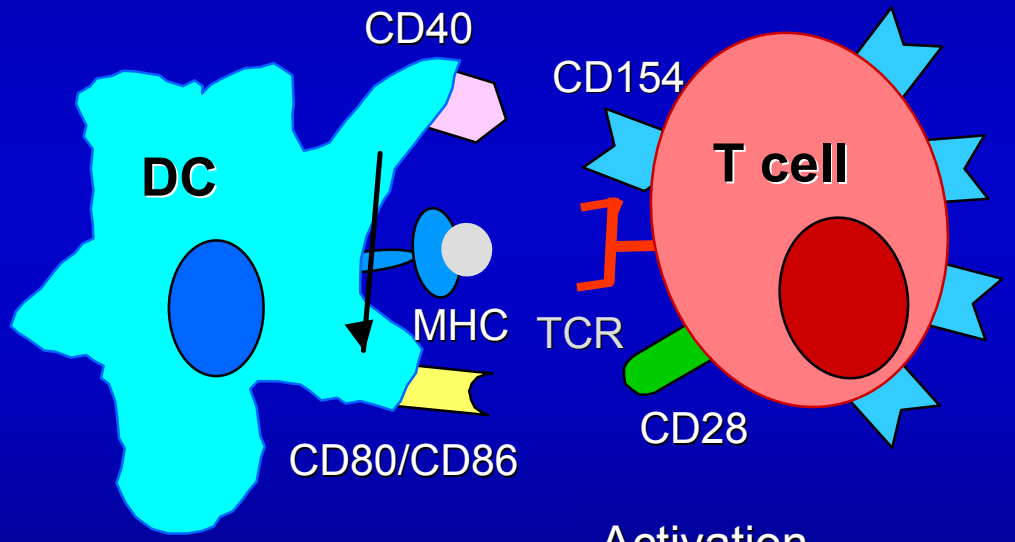
Agonizing a co-inhibitory pathway



Membrane-bound TNF-TNFR family members with costimulatory activity

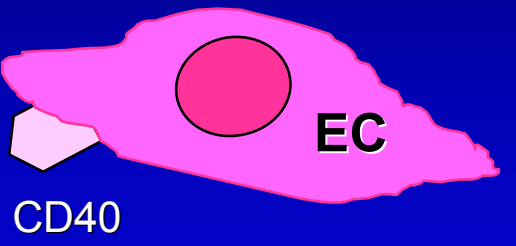


CD40-CD154 interactions in T cell-dependent immune responses

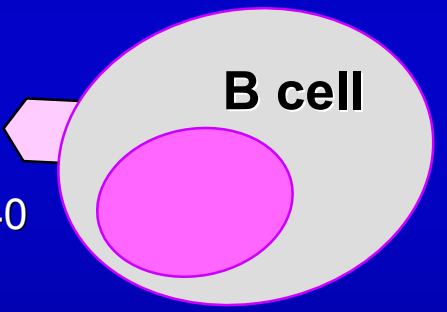


Increased
co-stimulation
CD80/CD86
CD54
IL-12

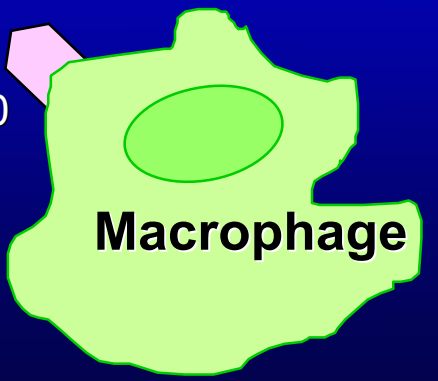
Activation
Sustained
clonal expansion



Expression of
adhesion molecules
CD54, CD106
CD62E



Proliferation
Ig class switching
CD80/CD86
expression



Effector functions
TNF-a
IL-1b
Nitric oxide

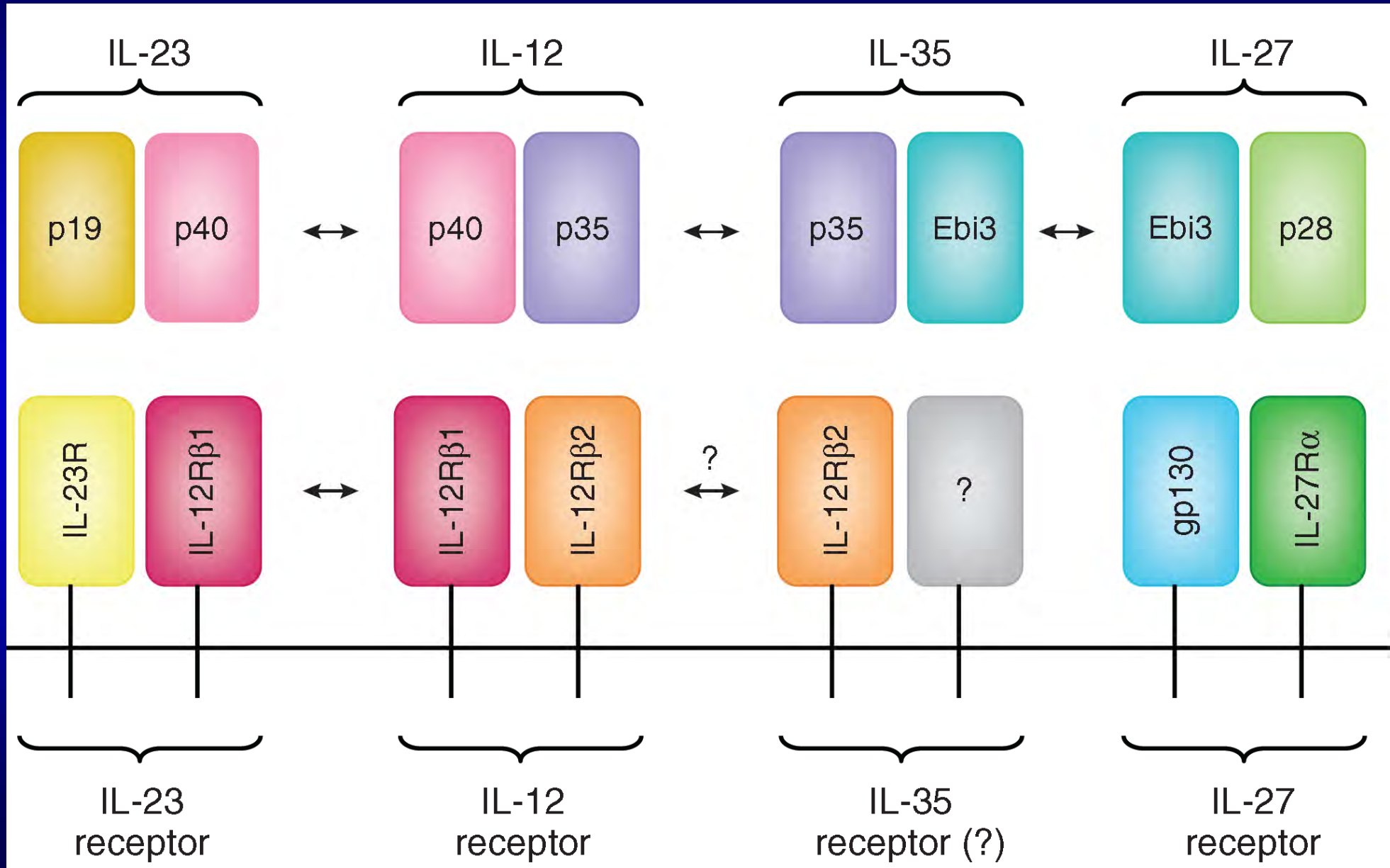
Anti-CD40L trials halted

- In October 1999 Biogen halted all anti-CD40L trials except in kidney transplantation, because o "thrombo-embolic events"
- Anti-CD40L stimulates activated platelets in vivo triggering endothelial cells to cause thrombus formation (Nature 1997)
- Halting anti-CD40L trials does not have implications for the validity of costimulation blockade in the treatment of allograft rejection or autoimmune diseases

Comparison of cytokine-based strategies in the treatment of autoimmune diseases

	Anti-TNF-α	IFN-β	IL-1Ra	IL-10	Anti-IL-12/IL-23p4
Pros	Validated clinical target. Effective in several autoimmune diseases (RA, Crohn's disease, psoriasis, spondiloarthopaties)	Most effective agent available for the treatment of MS	Decreases rate of bone erosion in RA patients	Multiple anti-inflammatory mechanisms of action	Targeting key Th1and Th17-inducing cytokines
Cons	Long-term side effects unknown. Etanercept does not work in Crohn's disease	Immunogenic. Efficacy so far demonstrated only in MS	Moderate efficacy. Side effects	Modest, if any, efficacy	Efficacy data in psoriatic arthriti psoriasis, CD
Latest	Several anti-TNF agents being tested in multiple additional autoimmune indications	Approved for RR and SP MS	Approved in the EU as a second-line treatment for RA, combined with MTX	Several phase II trials completed or ongoing	Phase II (MS, Crohn's disease psoriasis)

IL-12 family members



Palmer & Weber, Nature Immunol. 11:1, 2010

Anti-IL-12 agents in phase II clinical development

Agent	Description	Indications
ABT-874 (Abbott)	Anti-IL-12 p40 h mAb	CD, MS, psoriasis
CNTO 1275 (Centocor)	Anti-IL-12 p40 h mAb	Psoriasis, MS
STA-5326 (Synta)	Oral small molecule selective IL-12 inhibitor	CD, Psoriasis

Ustekinumab, a human interleukin 12/23 monoclonal antibody, for psoriatic arthritis: randomised, double-blind placebo-controlled, crossover trial

Alice Gottlieb, Alan Menter, Alan Mendelsohn, Yaung-Kaung Shen, Shu Li, Cynthia Guzzo, Scott Fretzin, Rod Kunynetz, Arthur Kavanaugh

Lancet 2009; 373: 633–40

Ustekinumab significantly reduced signs and symptoms of psoriatic arthritis and diminished skin lesions compared with placebo, and the drug was well tolerated.

Repeated subcutaneous injections of IL12/23 p40 neutralising antibody, ustekinumab, in patients with relapsing-remitting multiple sclerosis: a phase II, double-blind, placebo-controlled, randomised, dose-ranging study

Benjamin M Segal, Cris S Constantinescu, Aparna Raychaudhuri, Lilianne Kim, Rosanne Fidelus-Gort, Lloyd H Kasper, on behalf of the Ustekinumab MS Investigators*

Lancet Neurol 2008; 7: 796–804

Ustekinumab is generally well tolerated but does not show efficacy in reducing the cumulative number of gadolinium-enhancing T1-weighted lesions in multiple sclerosis.

Approaches to in vivo Treg manipulation

Cell therapy

Ex-vivo expansion ± treatment → reinfusion

In vivo enhancement

- Small molecules
- Biologicals

Direct

Via Tolerogen DCs

Adoptive transfer of Treg cells in autoimmune disease mode

Disease	Regulatory T cells	Mouse model	Effect on disease
Type 1 diabetes	BDC2.5 TCR-transgenic T_{Reg} cells (from NOD mice)	NOD.*Rag*$^{-/-}$ mice reconstituted with diabetogenic T cells	Prevention
		NOD.*Cd28*$^{-/-}$ mice (which lack T_{Reg} cells)	Prevention
		Diabetic NOD mice receiving syngeneic islets	Prevention
		NOD mice with new onset diabetes	Remission (60%)
	BDC2.5 TCR-transgenic T_{Reg} cells expanded by DCs *in vitro*	BDC2.5 TCR-transgenic mice treated with high doses of cyclophosphamide	Prevention
		NOD.SCID mice reconstituted with diabetogenic T cells	Prevention
		Pre-diabetic NOD mice	Prevention
		NOD mice with new onset diabetes	Remission (50%)
	Antigen-specific NOD T_{Reg} cells expanded *in vitro*	NOD.*Cd28*$^{-/-}$ mice	Prevention
	GAD65-specific T_R1 cells	NOD.SCID mice reconstituted with diabetogenic T cells	Prevention
Multiple sclerosis (EAE)	TCR-transgenic MBP-specific T_{Reg} cells	*Rag*$^{-/-}$ TCR-transgenic (MBP-specific) mice	Prevention of spontaneous disease
	T_{Reg} cells from naive C57BL/6 mice	C57BL/6 mice immunized with MOG_{35-55} peptide	Prevention of induced disease
	OVA-specific T_R1 cells	BALB/c mice immunized with mouse spinal-cord homogenate and with heat-killed *Mycobacterium tuberculosis*	Prevention of induced disease
	T_R1 cells induced by B7H1–immunoglobulin fusion protein plus immobilized CD3-specific antibody	C57BL/6 mice immunized with MOG_{35-55} peptide	Prevention of induced disease
Rheumatoid arthritis	T_{Reg} cells	Collagen-induced arthritis	Inhibited progression of early stage disease
Inflammatory bowel disease	T_{Reg} cells	SCID mice reconstituted with $CD45RB^{hi}$ cells	Reversal of established disease
	OVA-specific T_R1 cells	SCID mice reconstituted with $CD45RB^{hi}$ cells	Prevention
	Caecal-bacteria-specific T_R1 cells from C3H/HeJBir mice	SCID mice reconstituted with pathogenic T_H1 cells from C3H/HeJBir mice	Prevention
Systemic lupus erythematosus	Thymus-derived T_{Reg} cells	NZB × NZW mice	Control of autoimmunity

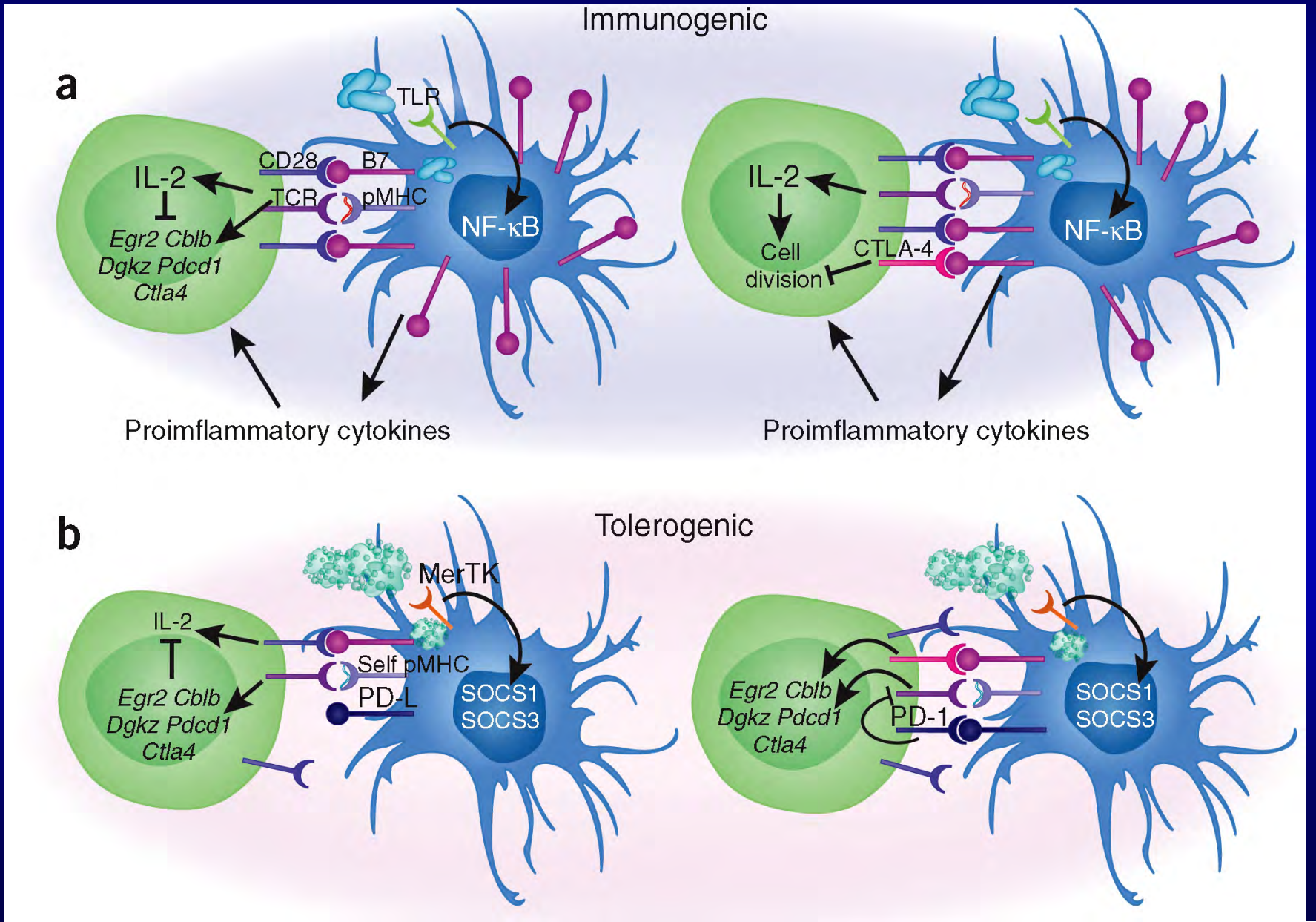
Roncarolo and Battaglia, Nat. Rev. Immunol. 7:585, 2007

Regulatory T cell-based immunotherapy in humans

Regulatory T-cell subset		Method	
Pros	Cons	Selection	Expansion/induction *in vitro*
FOXP3⁺CD4⁺CD25⁺ regulatory T cells			
• Circulating cells • Defined surface markers • Proven efficacy in several preclinical models	• Low numbers in the circulation • Multiple antigen specificity • Risk of pan immunosuppression	• Two-step procedure with magnetic beads to enrich for $CD4^+CD25^+$ cells • Further selection for low CD127 expression (?)	• Beads coated with CD3- and CD28-specific antib plus IL-2 and rapamycin • Antigen specific (?)
T regulatory type 1 cells			
• Inducible *ex vivo* • Antigen specific • Proven efficacy in several preclinical models	• Hard to purify	• Not feasible	• IL-10 with or without IFN • IL-10-treated dendritic c • Tolerogenic dendritic cel

Roncarolo and Battaglia, Nat. Rev. Immunol. 7:585, 2007

Immunogenic vs. Tolerogenic DCs



Induction of Tolerogenic DCs

- *Low costimulation*
- *High coinhibition*

- Anti-IL-12
- IL-10
- IL-13
- TGF-β
- CTLA4-Ig
- Anti-CD40L
- Salicylic acid
- Deoxyspergualin
- Glucocorticoids
- MMF
- Sirolimus
- $1,25(OH)_2D_3$

Tolerogenic Effects of Pharmacological Agents on Dendritic Cells

AGENT	EFFECTS ON DENDRITIC CELLS						
	Differentiation	Maturation	Costimulatory molecules	IL-12 Production	IL-10 Production	Allostimulatory capacity	NF-κB Activation
Acetylsalicylic acid		↓	↓	↓	↔	↓	↓
Butyric acid	↓	↓	↓				
Calcineurin inhibitors	↔	↓	↓	↓	↔	↓	↓
Deoxyspergualin		↓	↓	↓		↓	↓
Glucocorticoids	↓	↓	↓	↓	↔	↓	↓
N-acetyl-L-cysteine		↓	↓	↓		↓	↓
Mycophenolate mofetil	↓	↓	↓	↓		↓	
Sirolimus	↓	↓	↓	↓		↓	
Vitamin D3 analogs	↓	↓	↓	↓	↑	↓	↓

DC-T cell interactions: effects of VDR agonists