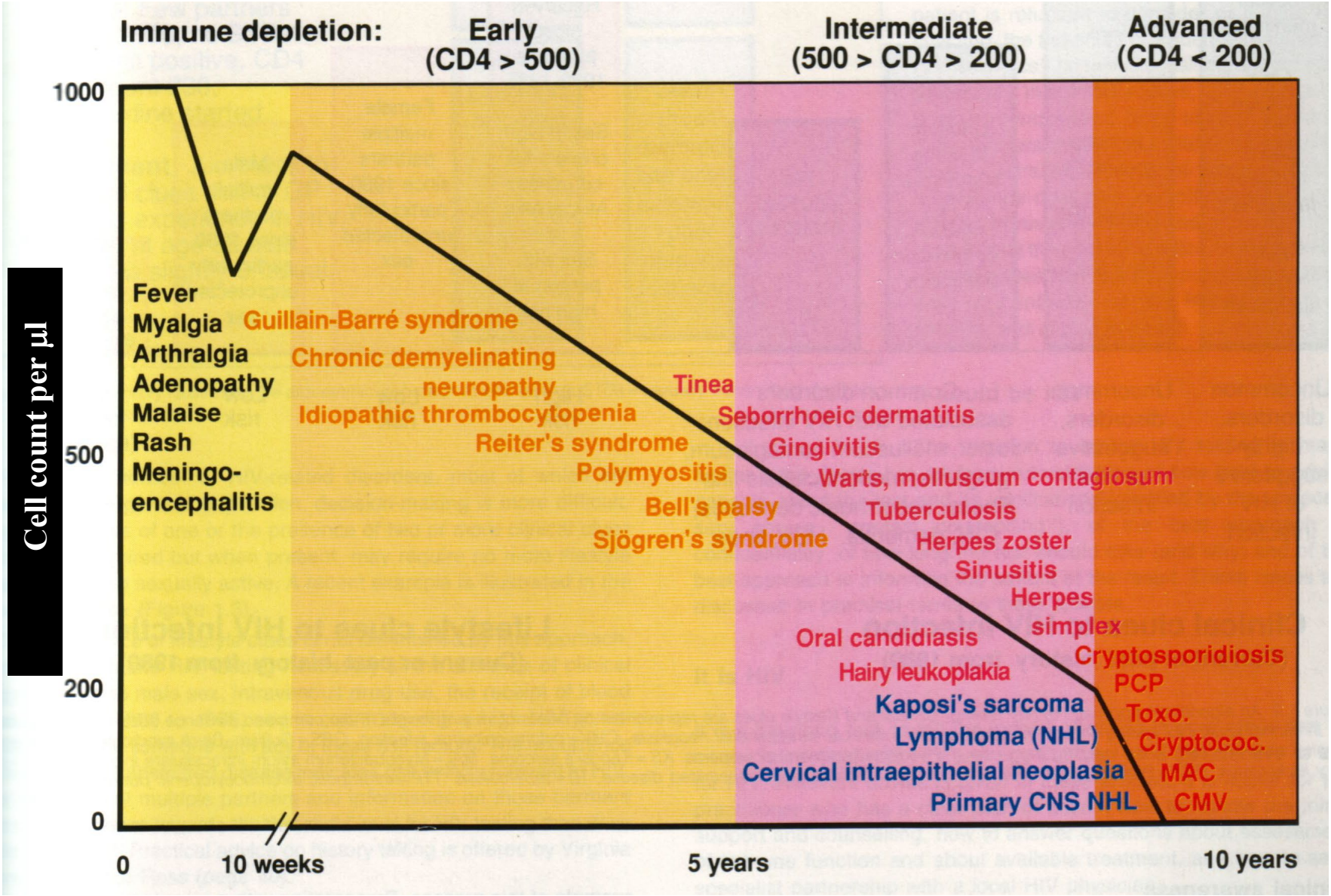
Topics in Immunology

Mina John

26th September 2020

Immunosuppression

HIV infection and Immune deficiency



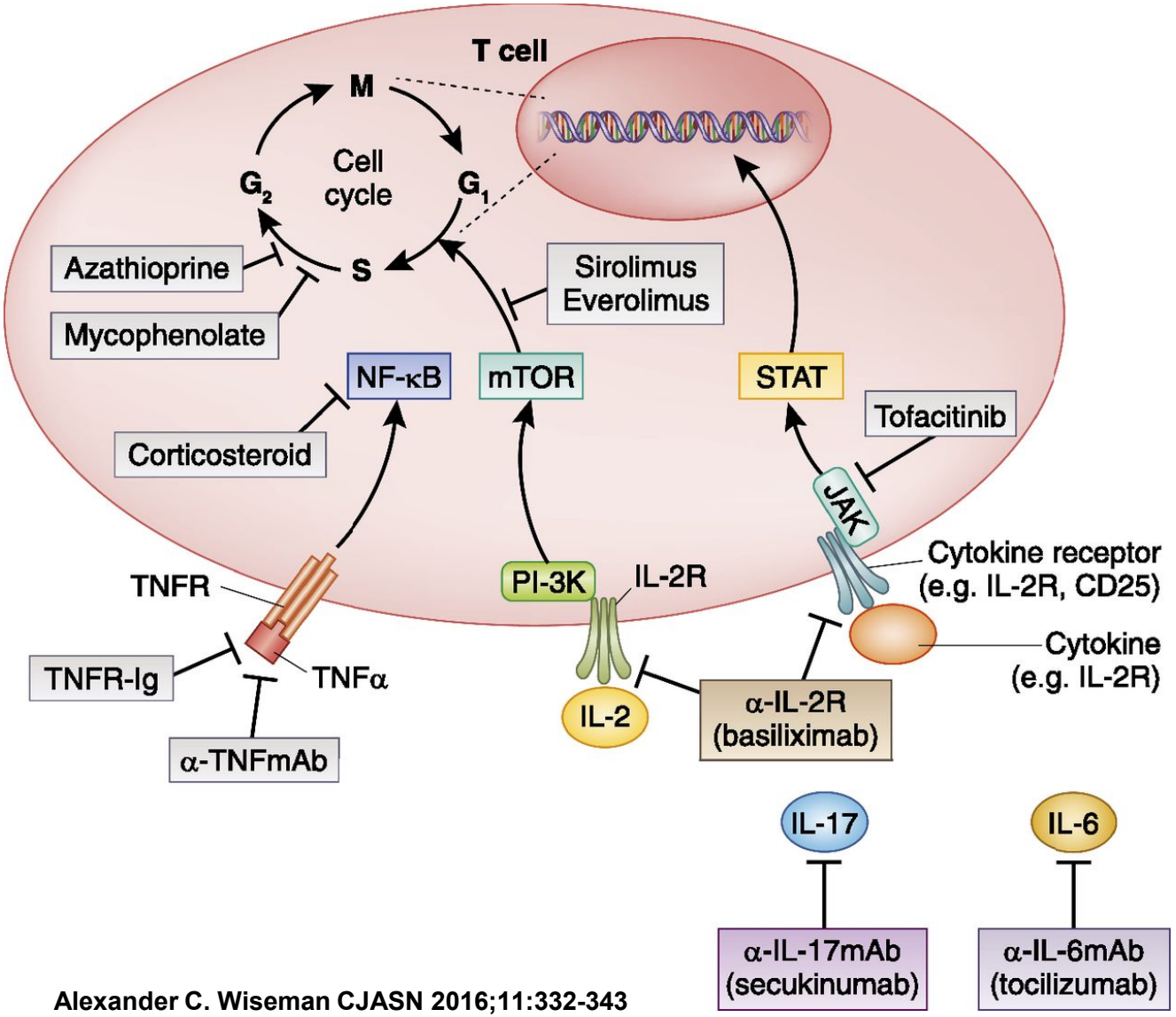
Principles of pathogen immunity

- **Specialisation**: The immune system has specialised functions.
  - Specific pathogens imply specific defects

Pathogen	Defect	
Encapsulated bacteria eg Strep. Pneumoniae, pseudomonas	Splenic function, B cells IgG, IgM	
Herpes viruses, intracellular bacteria, mycobacteria	T cells, NK cells, macrophages	

- **Redundancy**
- Disease is balance between **pathogen virulence versus immune response**
  - Low virulence organisms cause disease as immune response declines
  - Inflammation may be driven by excessive or dysregulated immune responses rather than the pathogen alone

Immunosuppressive agents targeting T cells



Alexander C. Wiseman CJASN 2016;11:332-343

Drug	Mechanism of action	Cells/factors affected	Severity of Immunosuppression	
Hydroxychloroquine	TLR7 antagonist Reduces immune activation		-	
Azathioprine/6 mercaptopurine	Inhibits nucleotide synthesis	T cell function>B cells	+	
Mycophenolate	Inhibits nucleotide synthesis	T cell function> B cells	+	
Methotrexate LD vs HD	Folate antagonist	T cell numbers and function	+	+ prolonged use + leukopenia
Cyclosporin/Tacrolimus	Calcineurin inhibitor	T cell function	+	
Cyclophosphamide	Alkylating agent	T and B cells numbers and function	+++	Cumulative dosing, Combined with other agents
Corticosteroids	Inhibitors cytokine production at transcriptional level	All lymphocytes Blunt inflammatory responses	+++	Cumulative dosing, combined with other agents

Important co-factors

- Age
- Diabetes mellitus
- Smoking/lung disease
- Alcoholism/liver disease
- Multiple co-morbidities/frailty

“Low risk vs high risk immunosuppression”

Low

- Prednisolone <20 mg/day for <2 weeks
- Inhaled or topical steroids
- Steroid injections (eg intra-articular)
- Physiological doses of maintenance steroids.

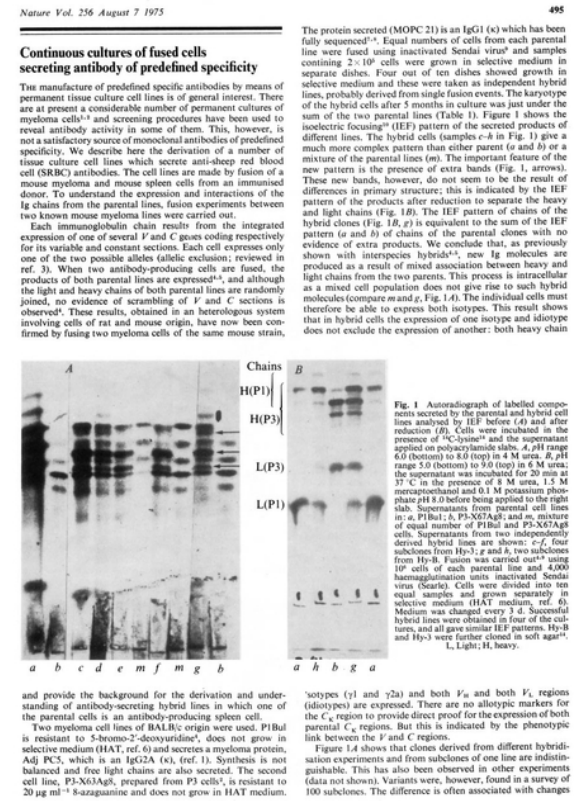
- sulfasalazine, hydroxychloroquine

- methotrexate ≤0.4 mg/kg/week,
- azathioprine ≤3 mg/kg/day,
- 6-mercaptopurine ≤1.5 mg/kg/day

High

- Prednisolone 20 mg/day for ≥2 weeks.
- Cyclophosphamide.
- Higher dose methotrexate.
- Transplant medications (eg cyclosporine and tacrolimus.
- Cancer chemotherapeutic agents.
- Biological DMARDs
  - aTNF-blockers (eg etanercept, adalimumab, certolizumab pegol, golimumab and infliximab).
  - lymphocyte-depleting agents (thymoglobulin or alemtuzumab) and B cell–depleting agents (rituximab).

Therapeutic MAbs



Nature Vol. 256 August 7 1975 495

Continuous cultures of fused cells secreting antibody of predefined specificity

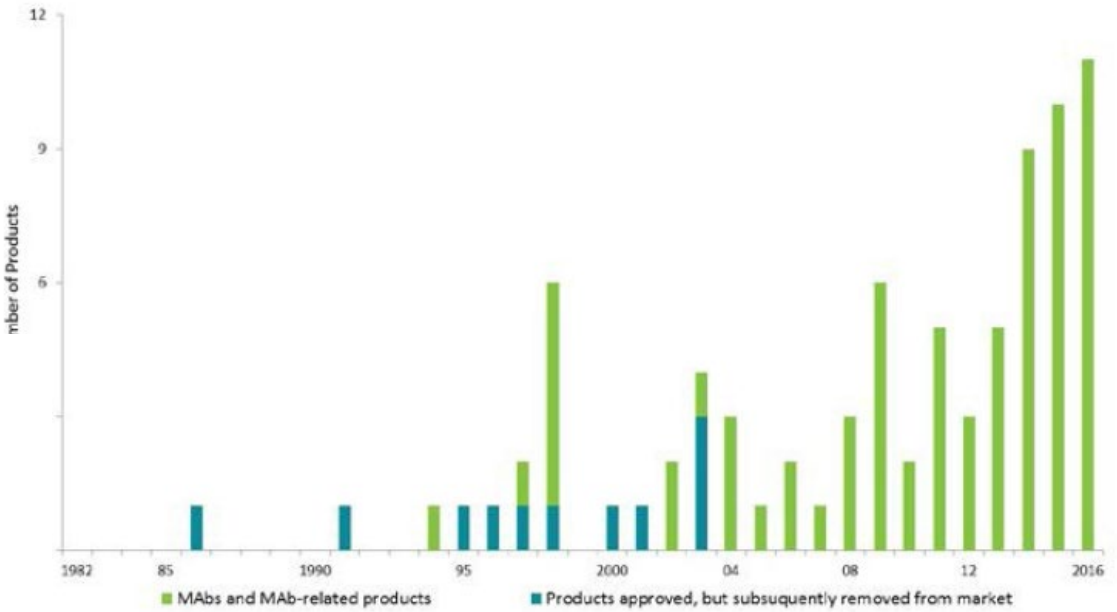
THE manufacture of predefined specific antibodies by means of permanent tissue culture cell lines is of general interest. There are at present a considerable number of permanent cultures of myeloma cells[1-3] and screening procedures have been used to reveal antibody activity in some of them. This, however, is not a satisfactory source of monoclonal antibodies of predefined specificity. We describe here the derivation of a number of tissue culture cell lines which secrete anti-sheep red blood cell (SRBC) antibodies. The cell lines are made by fusion of a mouse myeloma and mouse spleen cells from an immunised donor. To understand the expression and interactions of the Ig chains from the parental lines, fusion experiments between two known mouse myeloma lines were carried out.

Each immunoglobulin chain results from the integrated expression of one of several *V* and *C* genes coding respectively for its variable and constant sections. Each cell expresses only one of the two possible alleles (allelic exclusion; reviewed in ref. 3). When two antibody-producing cells are fused, the products of both parental lines are expressed[4-6], and although the light and heavy chains of both parental lines are randomly joined, no evidence of scrambling of *V* and *C* sections is observed[4]. These results, obtained in an heterologous system involving cells of rat and mouse origin, have now been confirmed by fusing two myeloma cells of the same mouse strain, and provide the background for the derivation and understanding of antibody-secreting hybrid lines in which one of the parental cells is an antibody-producing spleen cell.

Two myeloma cell lines of BALB/c origin were used. P1Bul is resistant to 5-bromo-2'-deoxyuridine[6], does not grow in selective medium (HAT, ref. 6) and secretes a myeloma protein, Adj PC5, which is an IgG2A (κ), (ref. 1). Synthesis is not balanced and free light chains are also secreted. The second cell line, P3-X63Ag8, prepared from P3 cells[7], is resistant to 20 μg ml^{-1} 8-azaguanine and does not grow in HAT medium. The protein secreted (MOPC 21) is an IgG1 (κ) which has been fully sequenced[1-4]. Equal numbers of cells from each parental line were fused using inactivated Sendai virus[8] and samples containing 2×10^5 cells were grown in selective medium in separate dishes. Four out of ten dishes showed growth in selective medium and these were taken as independent hybrid lines, probably derived from single fusion events. The karyotype of the hybrid cells after 5 months in culture was just under the sum of the two parental lines (Table 1). Figure 1 shows the isoelectric focusing[9] (IEF) pattern of the secreted products of different lines. The hybrid cells (samples *c–h* in Fig. 1) give a much more complex pattern than either parent (*a* and *b*) or a mixture of the parental lines (*m*). The important feature of the new pattern is the presence of extra bands (Fig. 1, arrows). These new bands, however, do not seem to be the result of differences in primary structure; this is indicated by the IEF pattern of the products after reduction to separate the heavy and light chains (Fig. 1*B*). The IEF pattern of chains of the hybrid clones (Fig. 1*B*, *g*) is equivalent to the sum of the IEF pattern (*a* and *b*) of chains of the parental clones with no evidence of extra products. We conclude that, as previously shown with interspecies hybrids[4-6], new Ig molecules are produced as a result of mixed association between heavy and light chains from the two parents. This process is intracellular as a mixed cell population does not give rise to such hybrid molecules (compare *m* and *g*, Fig. 1*A*). The individual cells must therefore be able to express both isotypes. This result shows that in hybrid cells the expression of one isotype and idiotype does not exclude the expression of another: both heavy chain isotypes (γ1 and γ2a) and both V_H and V_L regions (idiotypes) are expressed. There are no allotypic markers for the C_K region to provide direct proof for the expression of both parental C_K regions. But this is indicated by the phenotypic link between the *V* and *C* regions.

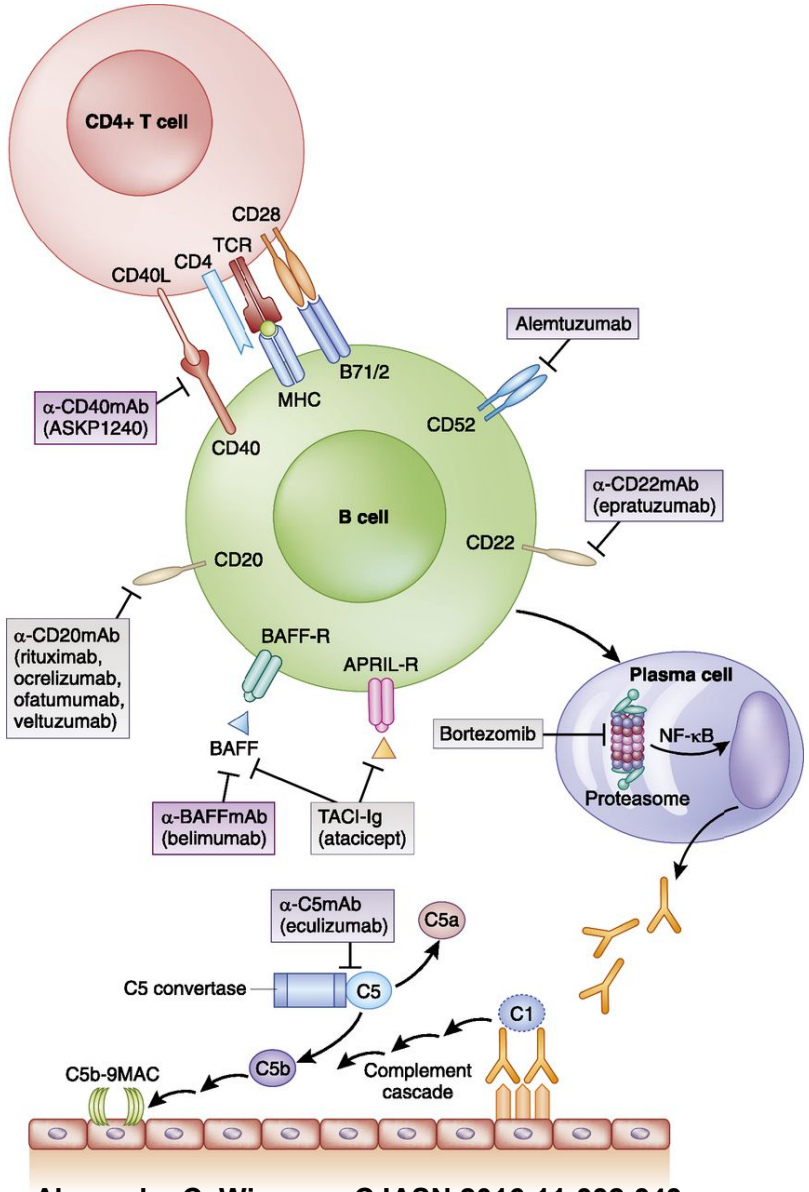
Figure 1*A* shows that clones derived from different hybridisation experiments and from subclones of one line are indistinguishable. This has also been observed in other experiments (data not shown). Variants were, however, found in a survey of 100 subclones. The difference is often associated with changes

Fig. 1 Autoradiograph of labelled components secreted by the parental and hybrid cell lines analysed by IEF before (*A*) and after reduction (*B*). Cells were incubated in the presence of ^{14}C-lysine[24] and the supernatant applied on polyacrylamide slabs. *A*, pH range 6.0 (bottom) to 8.0 (top) in 4 M urea. *B*, pH range 5.0 (bottom) to 9.0 (top) in 6 M urea; the supernatant was incubated for 20 min at 37 °C in the presence of 8 M urea, 1.5 M mercaptoethanol and 0.1 M potassium phosphate pH 8.0 before being applied to the right slab. Supernatants from parental cell lines in: *a*, P1Bul; *b*, P3-X63Ag8; and *m*, mixture of equal number of P1Bul and P3-X63Ag8 cells. Supernatants from two independently derived hybrid lines are shown: *c–f*, four subclones from Hy-3; *g* and *h*, two subclones from Hy-B. Fusion was carried out[1-3] using 10^6 cells of each parental line and 4,000 haemagglutination units inactivated Sendai virus (Searle). Cells were divided into ten equal samples and grown separately in selective medium (HAT medium, ref. 6). Medium was changed every 3 d. Successful hybrid lines were obtained in four of the cultures, and all gave similar IEF patterns. Hy-B and Hy-3 were further cloned in soft agar[14]. L, Light; H, heavy.



- 48 Mabs licensed in US/Europe
- >300 in development
- Humira (adalimumab) global sales > $12 billion: highest selling biopharmaceutical product ever.
- Market size ~$125 billion 2020

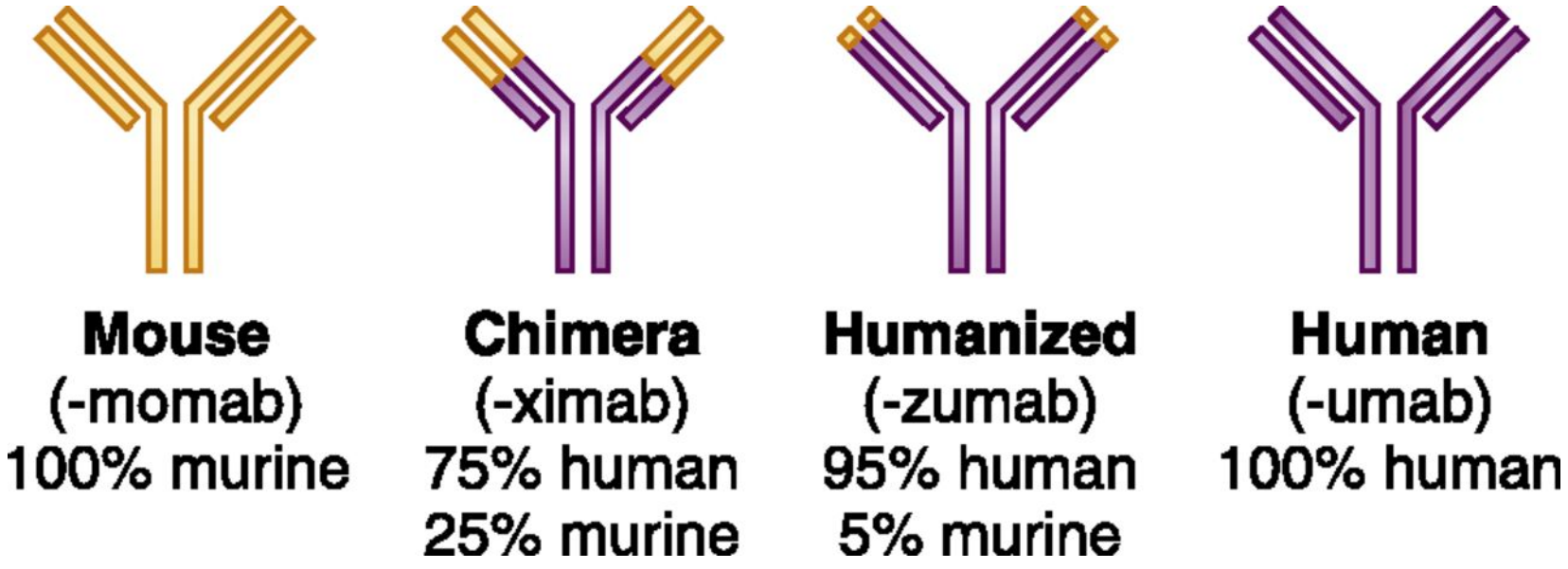
Immunosuppressive agents targeting T cell/B cell interaction, plasma cell function, and complement-mediated injury.



Alexander C. Wiseman CJASN 2016;11:332-343

CJASN

Schematic representation and nomenclature of mAbs in clinical use.



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CJASN

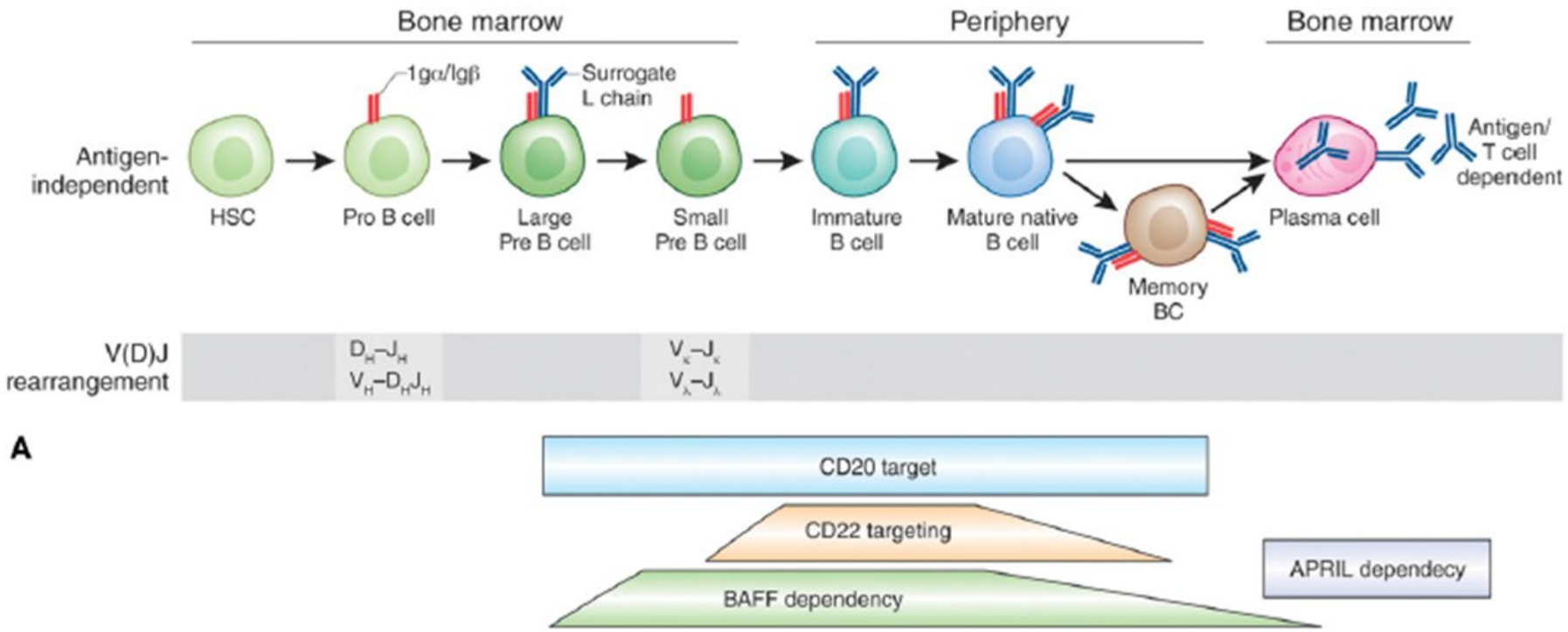
MAbs and risk of infection

- **B cell**
  - Rituximab
  - Ofatumumab
  - Inotuzimab (CD22)
- **T cell**
  - OKT3
  - Alemtuzimab
  - Basiliximab (Cd25)
- **T cell co-stimulation**
  - Abatacept
  - Belatacept
- **Leucocyte migration/egress**
  - Natalizumab
  - Fingolimod
  - Vedolizumab
- **Complement**
  - Eculizumab

- **Cytokine (neutralize cytokine action)**
  - Anakinra (IL1)
  - Canakinumab (IL1)
  - Rilonocept (IL1R)
  - Mepolizumab (IL5)
  - Reslizumab (IL5)
  - Dupilumab (IL4Ra)
  - Secukinumab (IL17A)
  - Ixekizumab (IgG4 IL17A)
  - Brodalumab (IL17R)
  - Ustekinumab (IL12/23R)
  - Infliximab (TNF)
  - Adalimumab (TNF)
  - Golimumab (TNF)
  - Etanercept (TNF)
  - Certolizumab pegol (TNF)

- **Anti-Cytokine (B cell-function)**
  - Tocilizumab (IL-6)
  - Siltuximab (IL-6)
  - Belimumab (sBlyS)
- **Small molecule kinase inhibitors**
  - Ibrutinib (BTK)
  - Idelalisib (PI3K)
  - Tofacitinib (JAK)
  - Ruxolitinib (JAK)

Rituximab and CD20+ cell depletion



Borner et al. 2018

Infection rate on rituximab (background RA)

Table 1 Patient characteristics at baseline*

	Rituximab+MTX All Exposure (n=3194)	Rituximab+MTX >5 years (n=627)	Placebo+MTX (n=818)
Age (years)	51.5	51.8	51.0
Female (%)	80.8	80.1	80.4
Disease duration (years)	8.3	11.1	7.1
Swollen joint count	20.0	21.5	17.7
Tender joint count	31.1	32.4	28.6
CRP (mg/dl)	2.83	3.37	2.83
ESR (mm/h)	47.1	46.1	45.2
DAS28-ESR	6.64	6.78	6.32
RF-positive (%)	77.6	85.3	81.3
Number of previous biologicals or DMARDs†	2.2	3.1	1.9
Number of previous biologicals	0.7	0.9	0.5
Baseline concomitant corticosteroids (%)	52.4	55.0	53.2

*Baseline characteristics were the observed mean values of the original baseline, where original baseline was the screening or baseline visit in the patient's original study.
†Excluding MTX.
CRP, C reactive protein; DAS28, disease activity score in 28 joints; DMARD, disease-modifying antirheumatic drug; ESR, erythrocyte sedimentation rate; MTX, methotrexate; RF, rheumatoid factor.

- Global Clinical Trial program
- >12000 patient years of observation
- >3000 patients with moderate-severe RA
- Up to 17 courses of rituximab
- Rates of infection not higher than placebo.

- Registry data: infection rate 3-5/100 person-years

Table 2 Summary of adverse events/100 pt-years

	Rituximab+MTX All Exposure (n=3194)	Rituximab+MTX >5 years (n=627)	Placebo+MTX (n=818)
Exposure (pt-years)	11 962	4418	1107
AEs/100 pt-years (95% CI)	263.10 (260.21 to 266.02)	254.12 (249.46 to 258.86)	315.43 (305.14 to 326.06)
SAEs/100 pt-years (95% CI)	14.40 (13.73 to 15.09)	14.30 (13.23 to 15.46)	13.82 (11.79 to 16.19)
Infections/100 pt-years (95% CI)	81.64 (80.04 to 83.27)	75.41 (72.89 to 78.02)	90.39 (84.96 to 96.17)
SIEs/100 pt-years (95% CI)	3.94 (3.60 to 4.31)	3.26 (2.77 to 3.84)	3.79 (2.80 to 5.13)

AE, adverse event; MTX, methotrexate; pt-year, patient-year; SAE, serious adverse event; SIE, serious infection event.

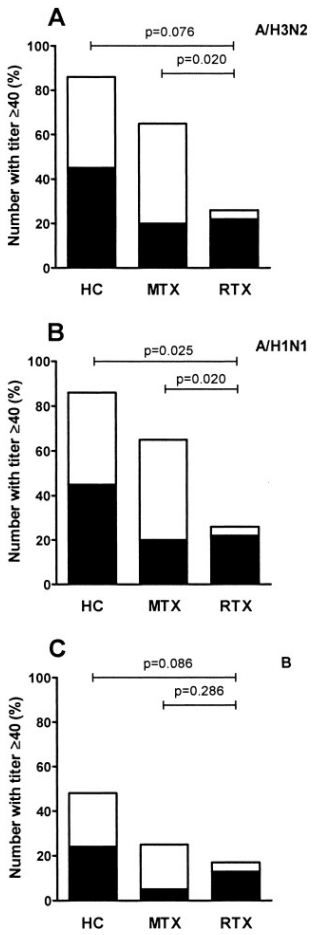
Van Vollenhoven et al Ann Rheum Dis 2013;72:1496–1502.

Rituximab induced Hypogammaglobulinemia

- Prevalence depends on underlying disease
  - Autoimmunity: <5%[1]
  - B cell malignancy: 20-43%
- Mean time to hypogammaglobulinemia 64 ± 23 months.
- Infections predominantly bacterial LRTI
- Risk factors;
  - multiple courses of Rx
  - Older age
  - Steroids, MMF/AZA, Methotrexate[1]
  - gammaglobulin levels <8g/L at baseline (HR 7.34, p = 0.003)[2]
  - methotrexate (HR 0.26, P = 0.03)[2].

1. *Van Vollenhoven et al. J Rheumatol 2010, 37:558*
2. *Boleto et al. Sem Arthritis and Rheum, 2018, 48 (2): 149-154*

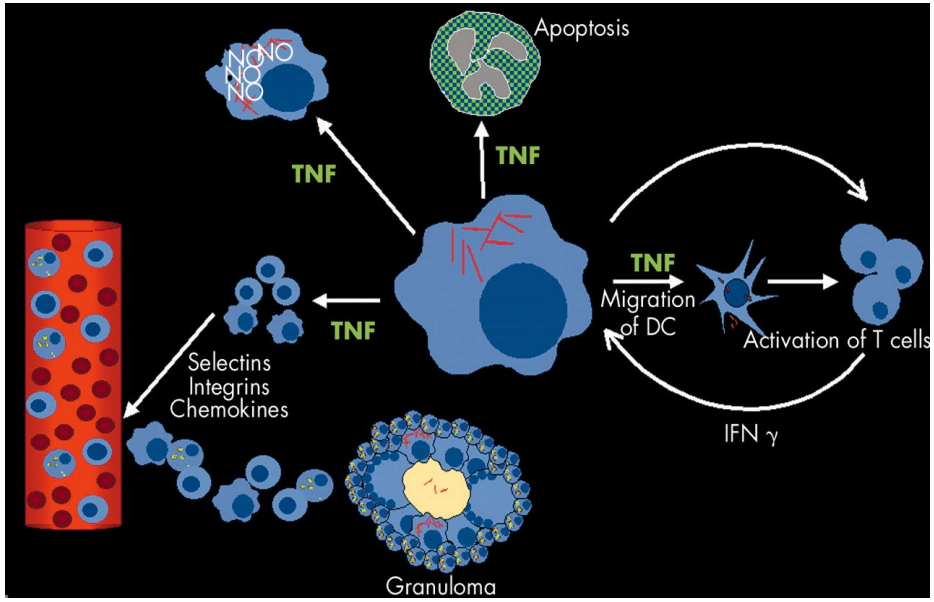
Rituximab, B cell deficiency and vaccine response

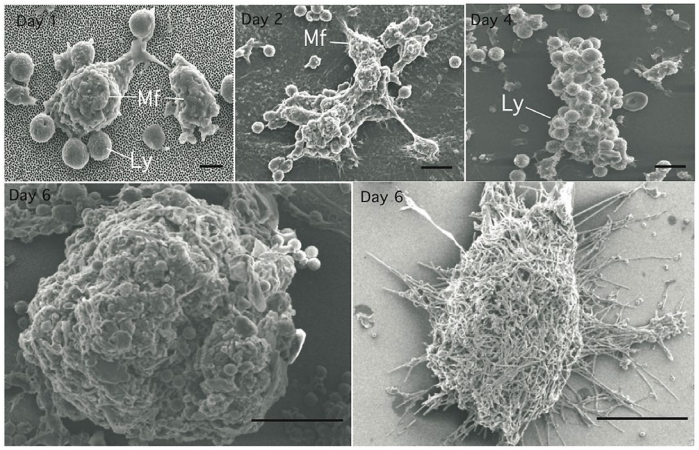


- B cell repopulation varies by disease [1]
  - 8 months RA
  - 9 months other CT disease
  - 26 months in GPA/MPA
- Reduced but protective responses [2,3]
  - Pneumococcal polysaccharide vaccine
  - Tet toxoid
  - Hepatitis A
  - Influenza
- Improved by later vaccination and B cell recovery [3]

1. *Theil et al. Arthritis Res Ther 2017, 19:101*
2. *Pescovitz et al. JACI 2011, 128:1295*
3. *Van Assen et al. Arthritis Rheum 2010,2:64*

TNFalpha





Intracelluar bacteria/fungi

- Mycobacterium *tuberculosis*
- NTD mycobacteria
- BCG
- Listeria species
- Nocardia
- Aspergillus *fumigatus*
- Toxoplasma *gondii*
- Cryptococcus *neoformans*
- Candida *albicans*
- Histoplasma *capsulatum*
- Coccidioides species

Viruses

- HBV
- VZV

Infections post TNFa inhibitors (eg infliximab, adalimumab)

- Incidence of infections requiring hospitalization : 2.9-8.2 per 100 patient-years in population-based studies

- German biologic registry study [1]
  - Adjusted for confounding factors
  - Incidence of serious infections increased by TNFa inhibitors
  - RR 1.8, 95% CI 1.2-2.7)
  - Most infection risk related to patient factors
    - Age
    - Comorbidities
    - Concomitant prednisone.
- In general responses to inactivated vaccines are not reduced

1. Strangfeld A et al. Ann Rheum Dis 2011; 70:1914.

Hepatitis-B reactivation https://www.hepatitisb.org.au/

All patients should have HBV serology including HBVcAb before immunosuppression to assess risk of reactivation and need for chemoprophylaxis

Table 1

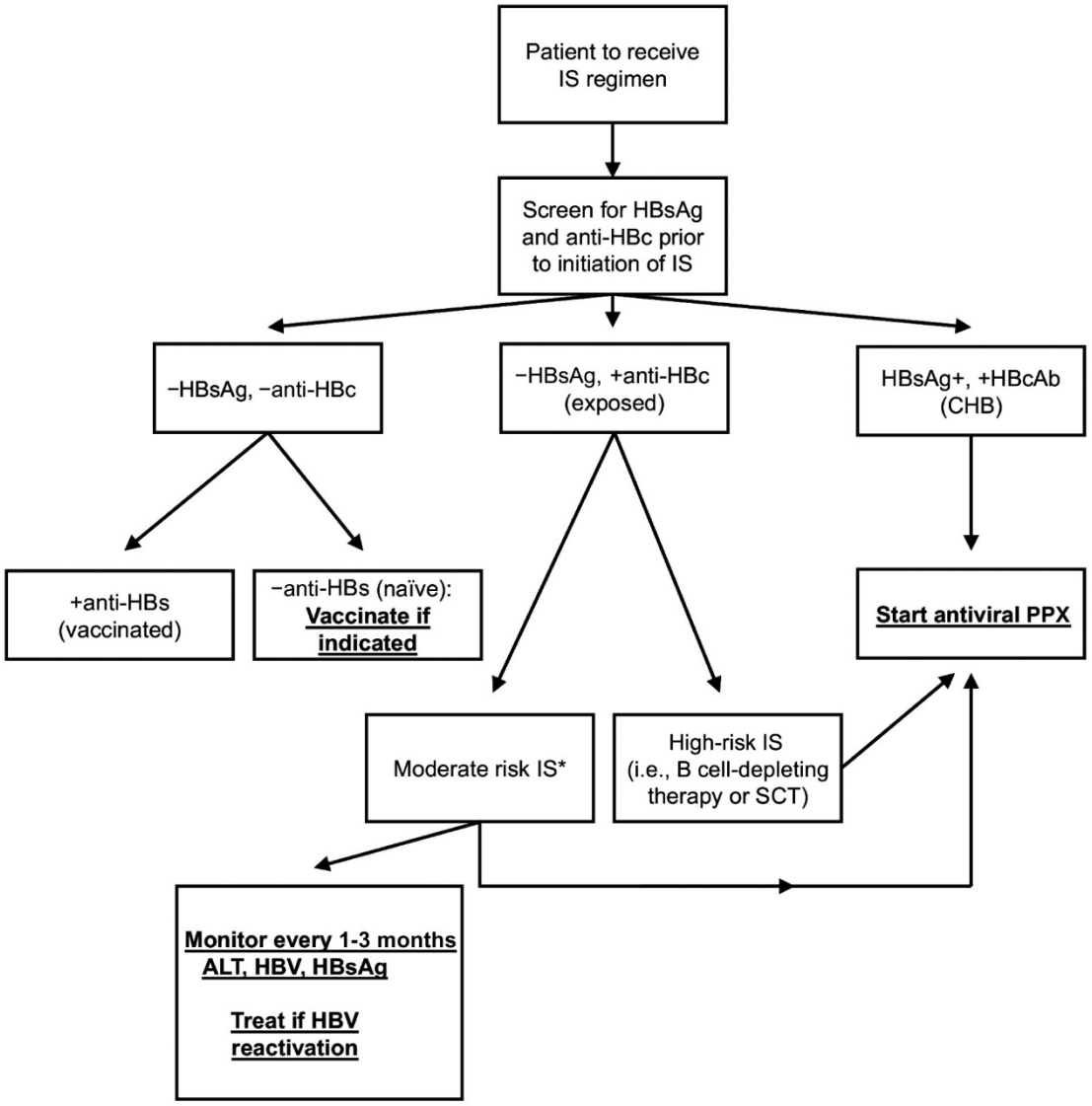
Immunosuppressive regimens known to increase risk of hepatitis B virus reactivation

HBsAg-positive	HBsAg-negative Anti-HBc positive
Corticosteroids	Anti-CD20 (*e.g.*, rituximab)
Anti-CD20 (*e.g.*, rituximab)	HSCT
HSCT	Anti-TNF
Anti-TNF	TACE for hepatocellular carcinoma
Anthracyclines	Methotrexate
TACE for hepatocellular carcinoma	
Methotrexate	
Ustekinumab	
Tyrosine kinase inhibitors	

HBsAg: Hepatitis B surface antigen; Anti-HBc: Antibody to hepatitis B core antigen; Anti-CD20: Antibody against CD20; Anti-TNF: Antibody against tumor necrosis factor; TACE: Transarterial chemo-embolization; HSCT: Hematopoietic stem cell transplantation.

Risk factors for reactivation
Intensity of immunosuppression/chemo regime
Longer duration of therapy
High baseline HBV DNA
HBVeAg+
Younger age
Male
Cyclical therapy

Seto, World Journal of Hepatology 2015



Duration

- 24 months after cessation if high risk
- 6-12 months if moderate risk

Clinical Liver Disease, Volume: 15, Issue: 4, Pages: 162-167, First published: 07 May 2020, DOI: (10.1002/cld.883)

Immunosuppression: Preventative care

- **Screening:** HIV serology, HepB serology, Latent TB screening, +/-VZV IgG , assessment of vaccine history and requirements

- **Pre-treatment vaccinations:**
  - Inactivated: ideally given at least 2 weeks before for maximal immunogenicity (esp rituximab
    - ll13vPPV + 23vPPV prime-boost
    - Flu vaccine
    - HPV
    - Others HAV, HBV, Meningococcal, Haemophilus as per specific risk assessment
  - Live vaccines: **must be given at least 4 weeks prior to commencing higher risk immunosuppression to prevent live vaccine dissemination
    - MMR,
    - Varicella/Zoster vaccines if indicated

- **If already on Immunosuppression**
  - Inactivated vaccines can be given as feasible but may have reduced response (esp Ritux): individualise decision on withholding/deferring immunosuppression
  - Low risk : Zostavax can be given but other live vaccines contraindicated
  - **High risk: All live vaccines contraindicated during and for a variable period after cessation**

General Precautions

- General hygiene measures
- Food hygiene
- Dietary precautions: unpasteurized milk, soft cheeses, undercooked chicken, eggs etc
- Travel**
- Covid-19:
  - Important to continue treatment to optimise underlying disease
  - General prevention measures still apply
  - Limited studies in this group
  - ASCIA https://allergy.org.au

Investigating for Immunodeficiency

Key warning signs

1. Frequent infections requiring treatment including:
    1. Serious middle ear /sinus infections without allergies (>2 per year)
    2. Pneumonias (>1 for >1 year)
    3. Chronic suppurative sinus or lung disease, bronchiectasis
2. Infections caused by low virulence or opportunistic types of organisms
3. Infections in unusual places eg perianal, deep organ abscesses
4. Infections that don't respond to treatment as normally expected,
    1. Refractory to antibiotic treatment
    2. Rapid recurrence of infections after ceasing antibiotic treatment
    3. Persistent unexplained oral thrush
    4. Severe infections (e.g. meningitis, osteomyelitis, pneumonia) requiring IV antibiotics
5. +Weight loss in adults
6. A family history of immunodeficiency or abnormal infections,
7. Autoimmune cytopenia, Granulomas, Chronic enteropathy, Unexplained hepatomegaly or splenomegaly
8. Non-response to vaccinations*

Investigations

- Microbiology to establish the organisms**
  - Encapsulated bacteria may indicate humoral immunodeficiency
- Consider general risk factors: Diabetes, renal and liver function, history of vaccination and vaccine response
- FBC
- Serum immunoglobulins IgG, IgM, IgA

Autoimmune disease “screening”

Autoimmunity Classification

Systemic

Systemic Lupus Erythematosis (SLE)

Systemic Sclerosis spectrum

Sjogrens disease

MTCD

Rheumatoid Arthritis

Organ-specific

Type I Diabetes Mellitus

Autoimmune thyroid disease

Autoimmune liver disease

Celiac Disease

Pernicious anaemia

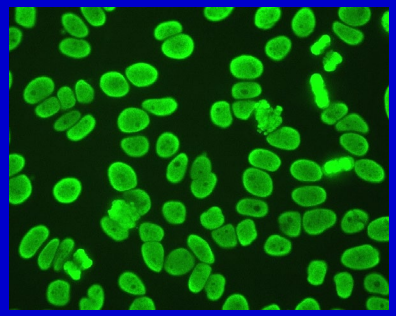
Inflammatory myositis/ILD

Features of most systemic autoimmune rheumatic diseases

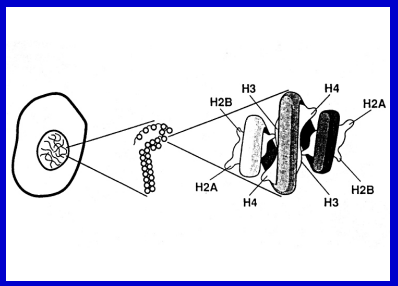
- Multiple sites of involvement
- Epidemiology- female preponderance, younger age of onset
- Arthropathy
  - Inflammatory nature
  - multiple, small joints
  - symmetrical
- Skin involvement
  - Photosensitivity

ANA Pattern relates to the specific antigen(s) bound by antibody

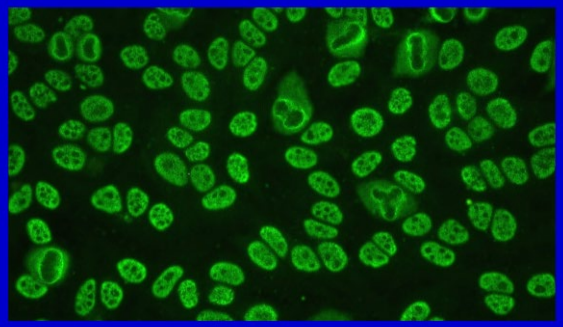
ANA Homogeneous: anti-histone or anti-DNA



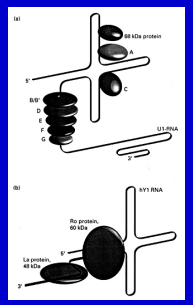
Chromatin,nucleosomes, histones, DNA



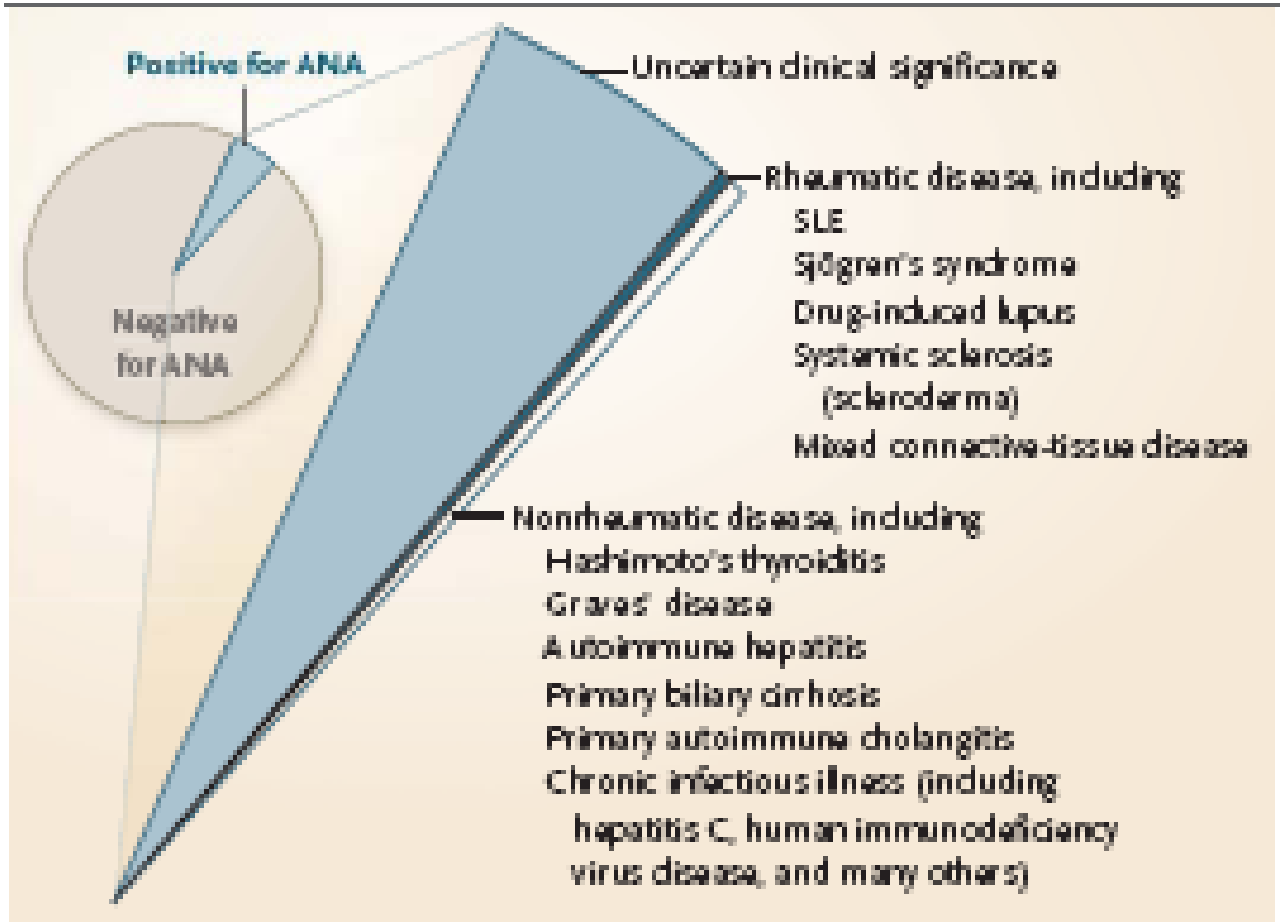
ANA speckled: anti U1 RNP



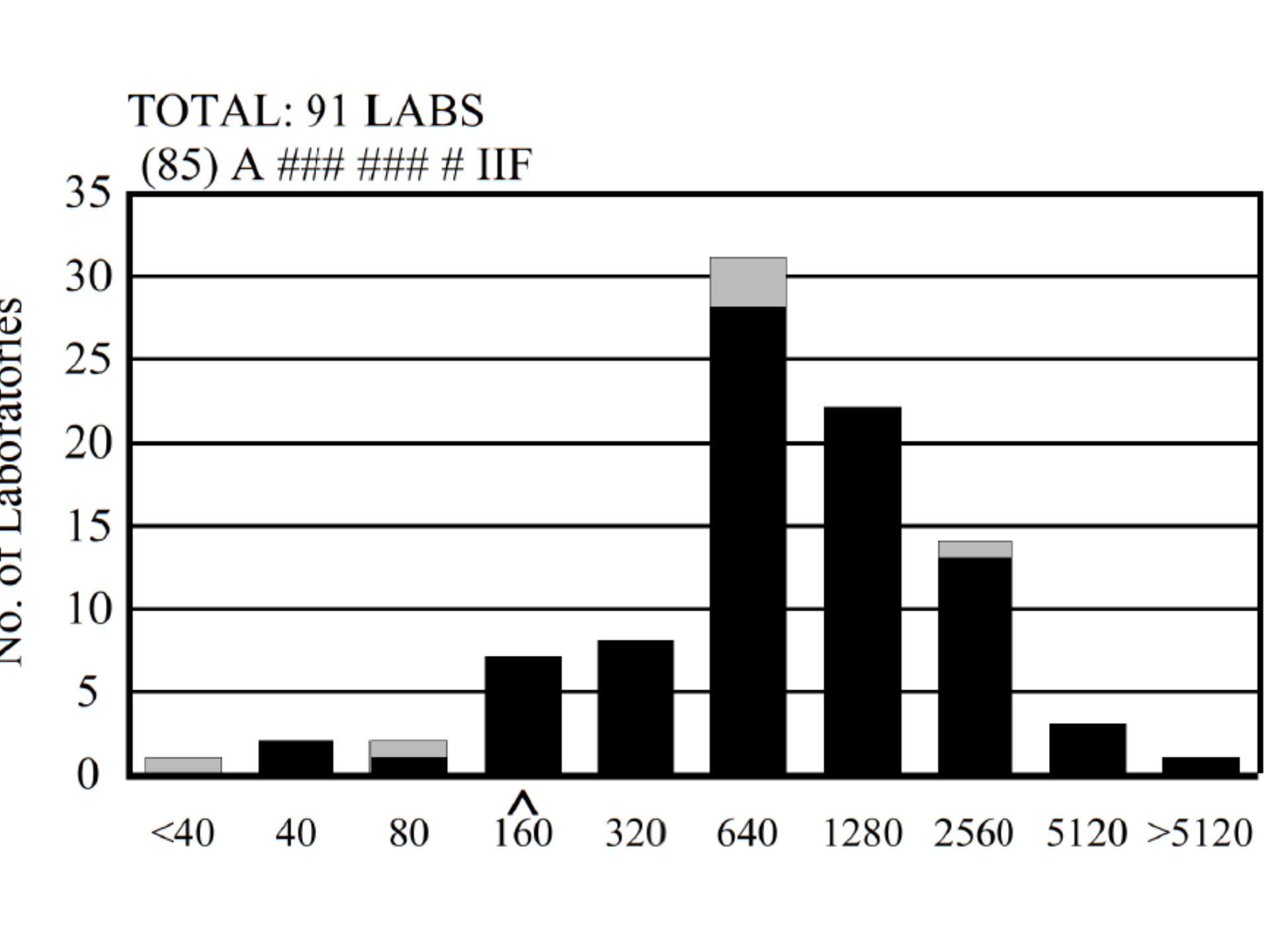
Soluble nuclear RNA-protein particles - U_1RNP, Sm, Ro, La



ANA in a hypothetical population



Shmerling RH. N Engl J Med 349;16:1499-1500 September 2005


TOTAL: 91 LABS
(85) A ### ### # IIF
No. of Laboratories
35
30
25
20
15
10
5
0
<40
40
80
160
320
640
1280
2560
5120
>5120

ANA occur in:

All pts with;

SLE
Neonatal LE
MCTD

Most patients with;

Drug LE
Sjogrens disease
Scleroderma spectrum
Polymyositis/DM

Many pts with;

Autoimmune thyroid disease
Coeliac disease
Chronic infections
IBD
autoimmune hepatitis
PSC
Chronic infections
Microcytic/lymphocytic colitis
Chronic urticaria
Advanced age

High titre moderate/low

Specific pattern, pattern homogenous,

+ENA or anti-ds no ENA or ds DNA

SLE : ANA Associations

Antigen	Clinical Associations	Prevalence (%)
Double stranded DNA	Renal disease, marker for disease activity	40-60%
Smith Antigen (Sm)		20%
Ro/SSA	Subacute cutaneous lupus, photosenitivity, neonatal lupus, Sjogrens	40%
La/SSB	Low prevalence of renal disease, Sjogrens syndrome	10-15%
Ribonuclear protein (U1-RNP)	Mixed connective tissue disease	30-40%
Phospholipids	Hypercoagulable states, thrombocytopenia, miscarriages, verrucous endocarditis	30%
Histones	Drug related SLE (not specific)	
Ribosomal P	Psychosis and depression	10-40%

Irrelevant ANA: DFS-70 antibodies

- A diffuse fine speckled pattern is prevalent in healthy people but not in rheumatic diseases.
- The molecular target has been cloned.
- Peptides representative of this particle can be used to absorb these antibodies from serum, making the ANA test more specific for disease
    - M Mahler , J G Hanley and M J Fritzler Autoimmunity Reviews 11; 9:642-645 2012

Vasculitis “screening”

- Most autoimmune serological tests are only relevant to a subset of systemic **small vessel vasculitis** syndromes
  - ANCA-associated vasculitides such as GPA, EGPA, MPA- ANCA
  - Cryoglobulinemic vasculitis- HCV, HBV, paraproteins, RhF
  - SLE or RA associated- CCP antibodies, ANA
- Inflammatory markers- CRP, ESR, Ferratin, RhF
- The rest is clinical/imaging and histopathology!

Summary

- ANA is a screen for a small group of uncommon ANA-associated systemic autoimmune diseases with common non-specific symptoms
- Has near 100% negative predictive value- use to exclude
- Will have more false positives at lower titre and low pre-test probability
- If moderate to high titre or specific pattern is shown- follow with more specific tests eg ENA, dsDNA, myositis panel etc