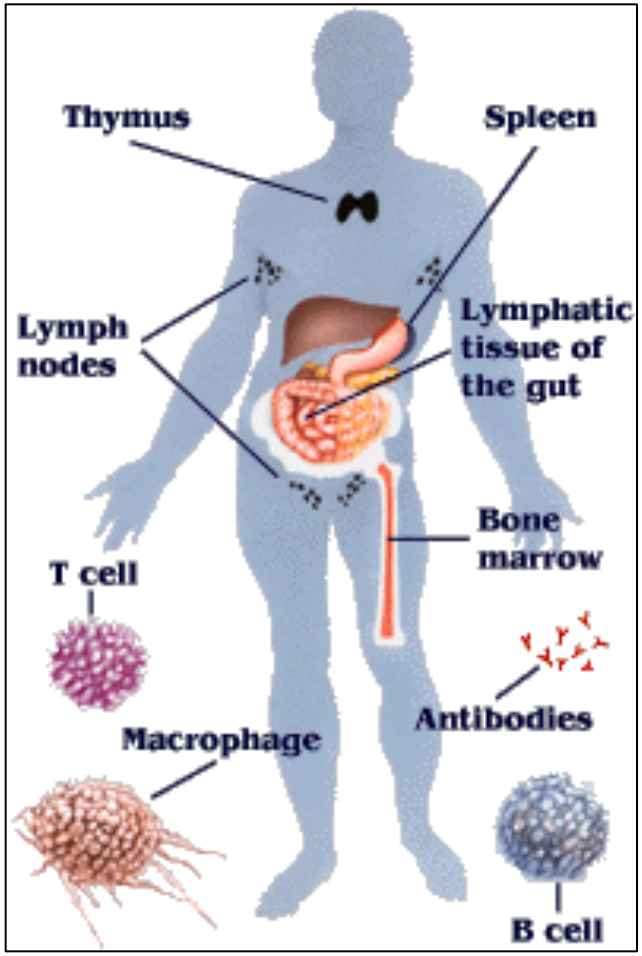
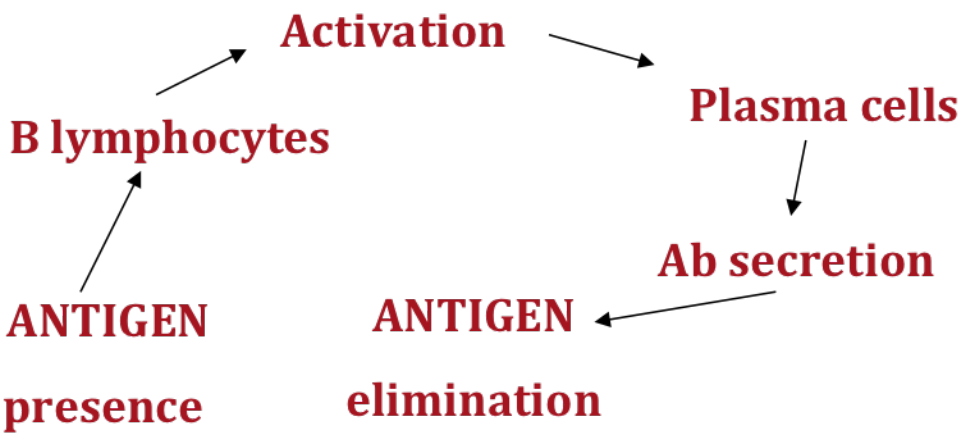
Human antibodies

General informations



- Innate Immunity (natural)
  - Humoral
  - Cell-mediated
- Acquired Immunity (specific)
  - Humoral
  - Cell-mediated

Antibodies are the most important component of humoral immune system.



Glossary

Antibody (anti-foreign body) is a glico-protein produced by a white cell (B lymphocyte and plasma cells).

Antigen (antibody generating substance) is any agent, such as a chemical or microorganism that is recognized by the antibody.
Not all antigens are immunogens (e.g hapten).

Immunogen: Any substance to which an animal responds by making antibodies. All immunogens are antigens.

Immunogenicity

Ability of a molecule to induce an immune response

Proteins, peptides, carbohydrates, nucleic acids, lipids

Must be larger than 3000-5000 daltons - if not…..

Antigen (hapten) → Carrier protein

e.g. BSA, Thyroglobulin

Carbodiimide

Glutaraldehyde

MBS–Heterobifunctional reagents

Glossary

Antibody (anti-foreign body) is a protein produced by a white cell (B lymphocyte).

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Epitope: the part of a target to which an antibody binds, also known as an antigenic determinant

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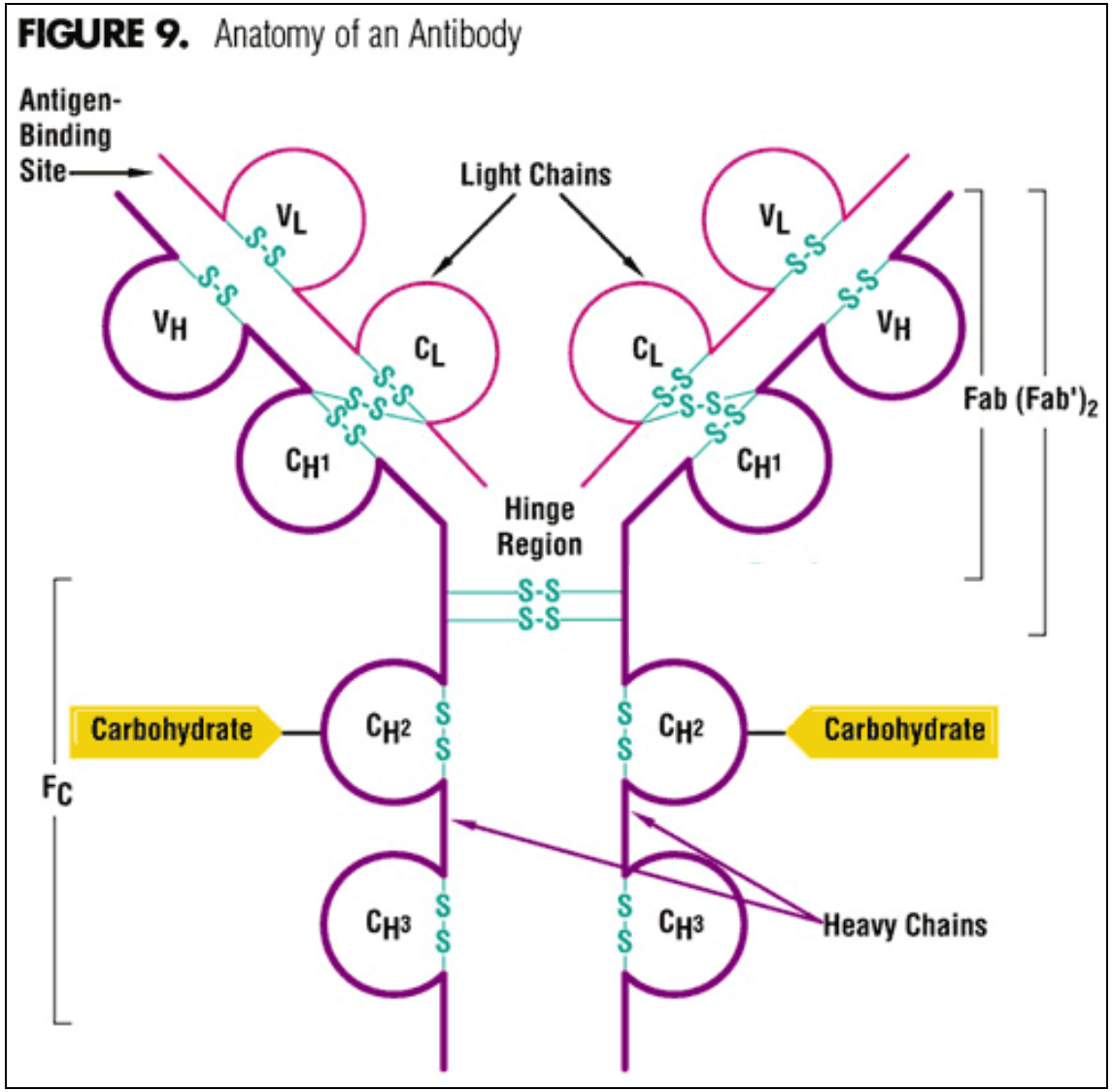
Antigen binding site - relatively small region of an antibody that binds to the antigen.

ANATOMY OF AN ANTIBODY



2 HEAVY CHAINS

1 VARIABLE DOMAIN (VH)

3 CONSTANT DOMAINS (CH1-2-3)

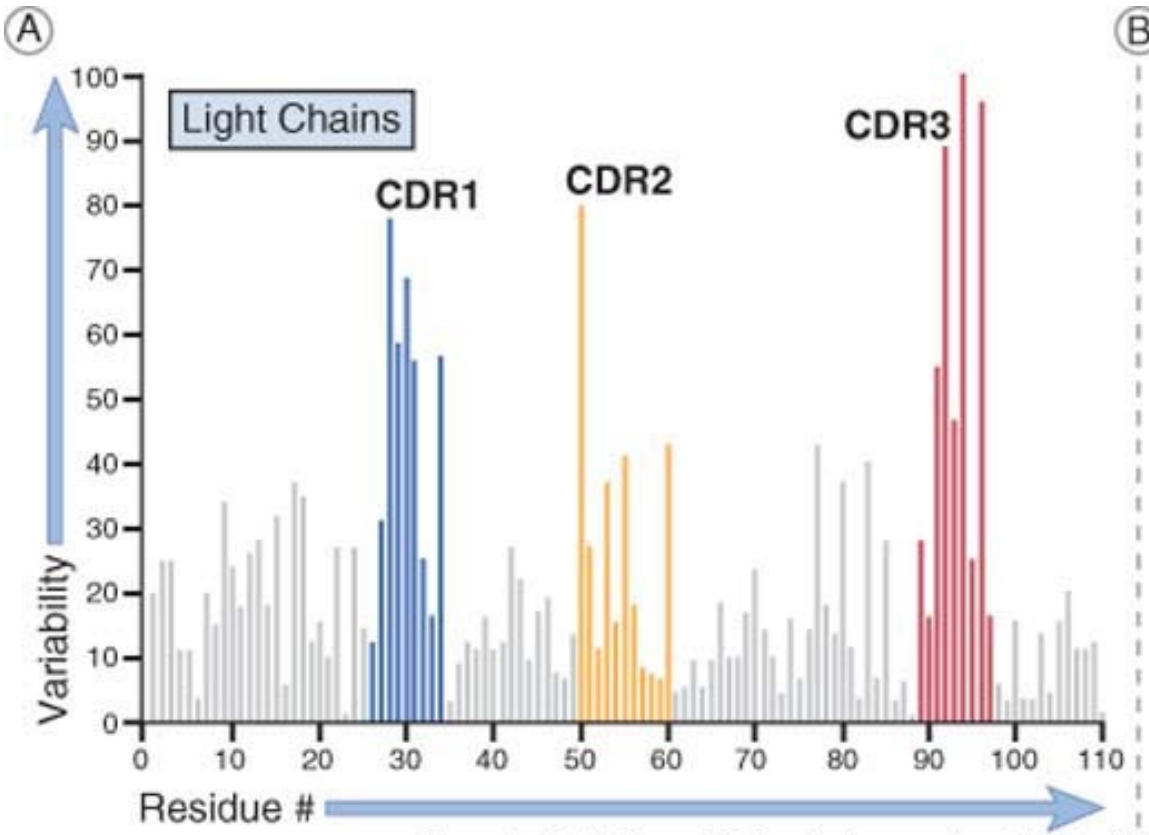
2 LIGHT CHAINS

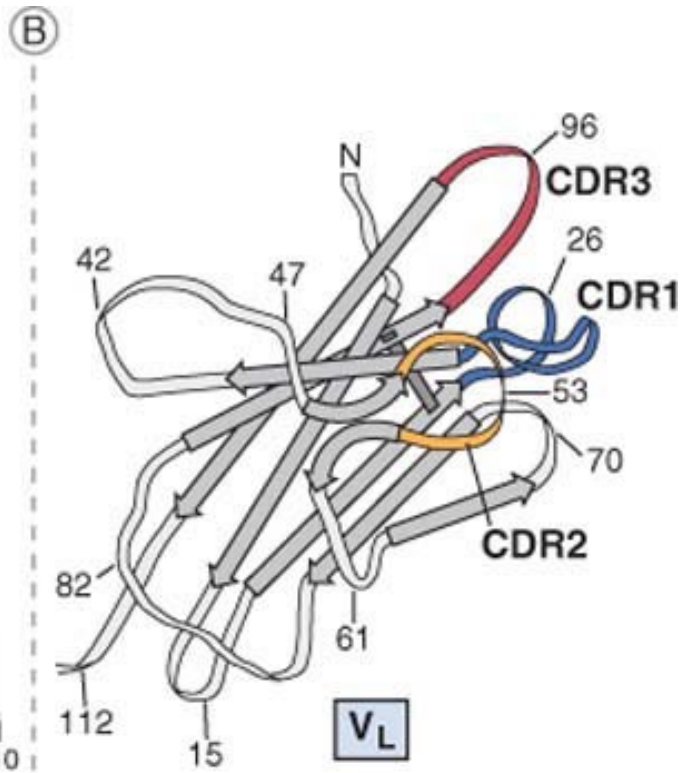
1 VARIABLE DOMAIN (VL)

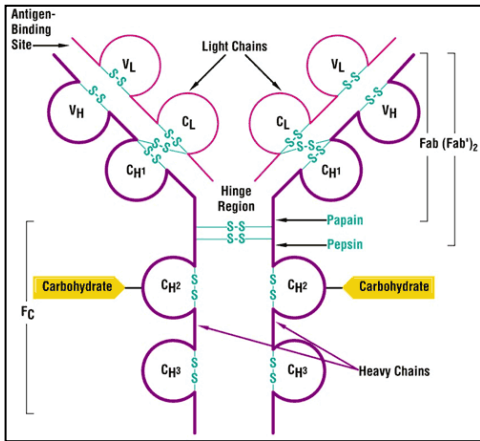
1 CONSTANT DOMAINS (CL)

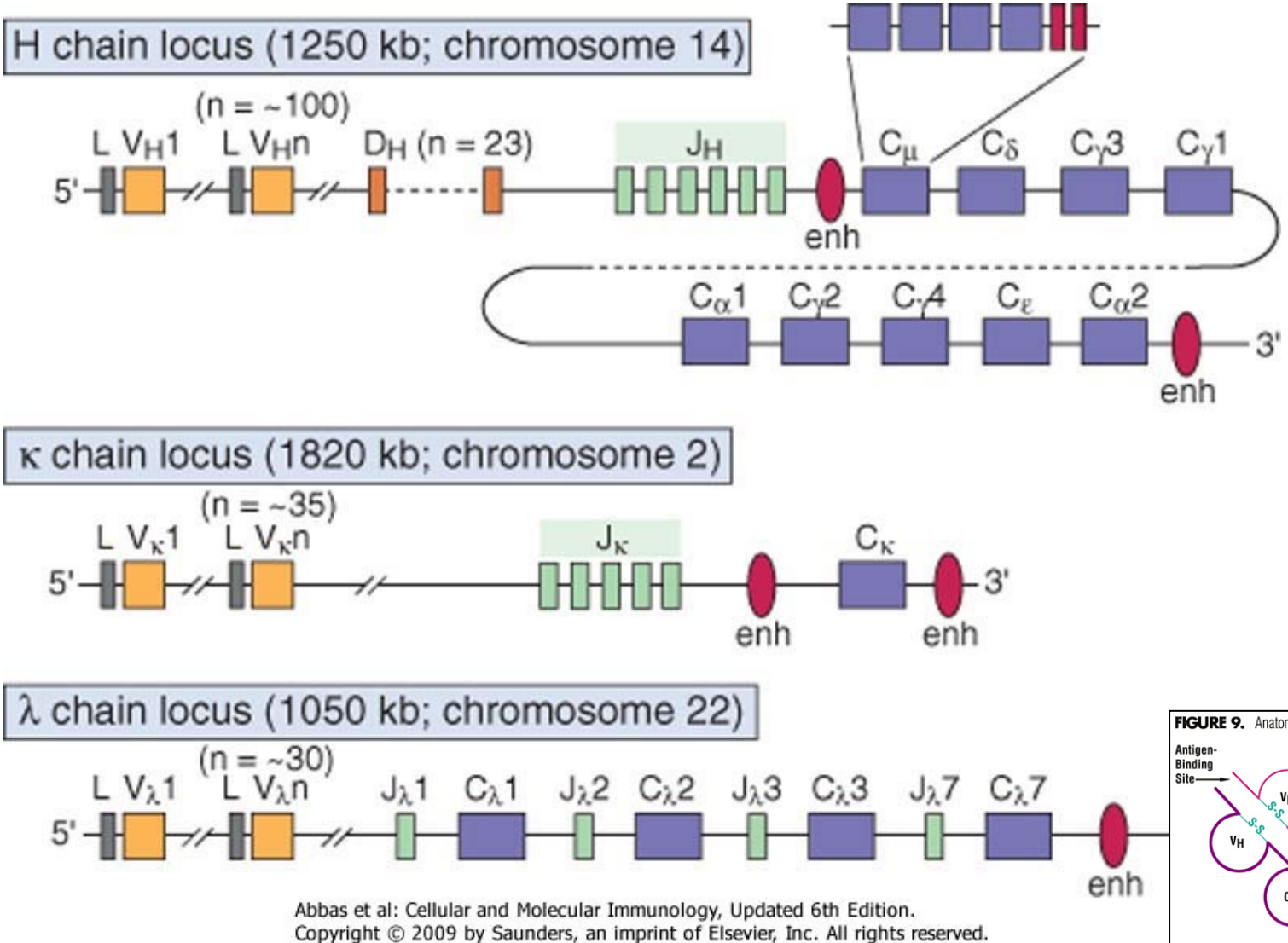
(k or λ)

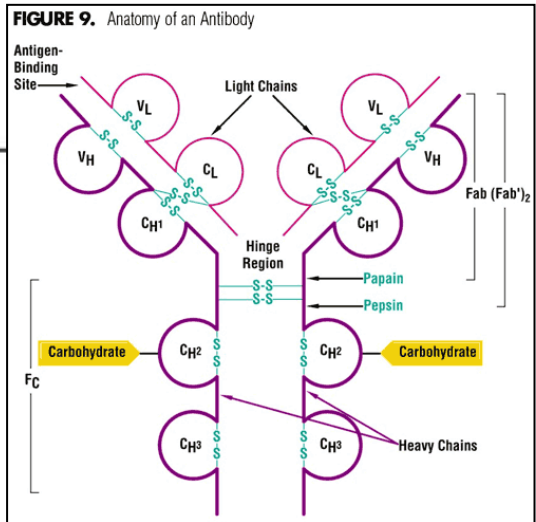
Aminoacid variability in antibody sequence











IG gene rearrangements - the LEGO approach

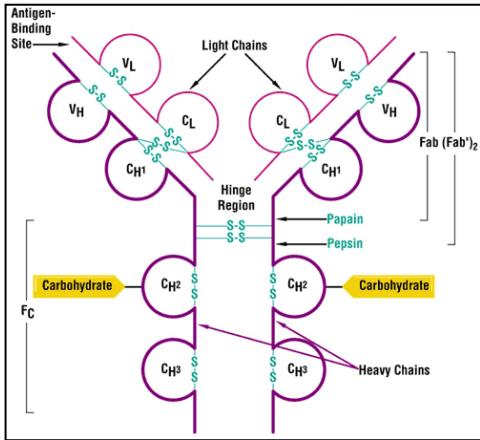
150 FUNCTIONAL IG GENES

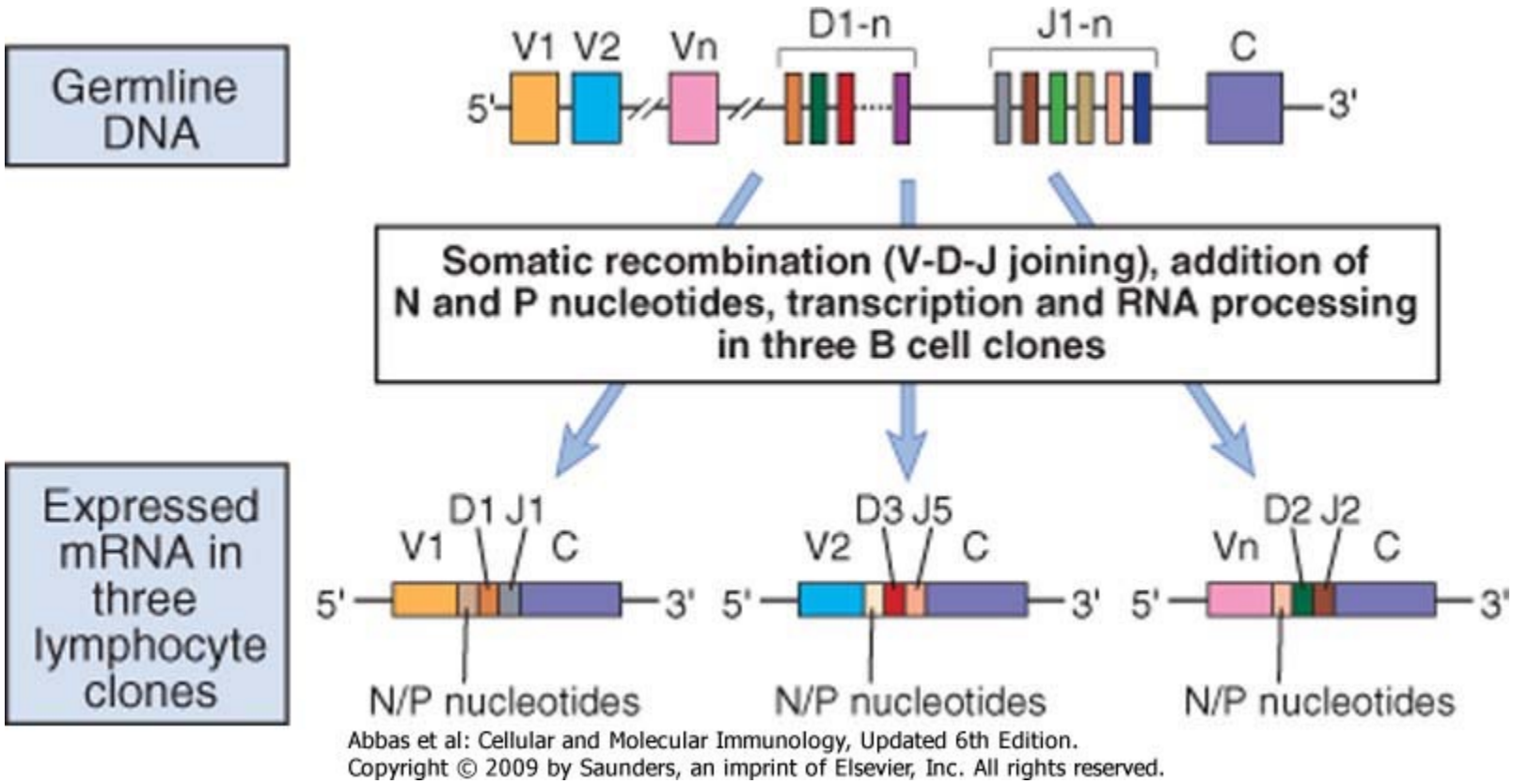
HEAVY CHAIN

6300 POTENTIAL RECOMBINATIONS

LIGHT CHAIN

185+165 POTENTIAL RECOMBINATIONS





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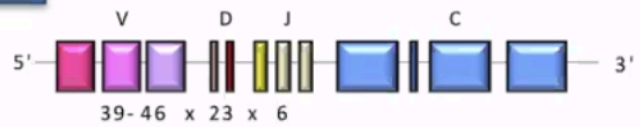


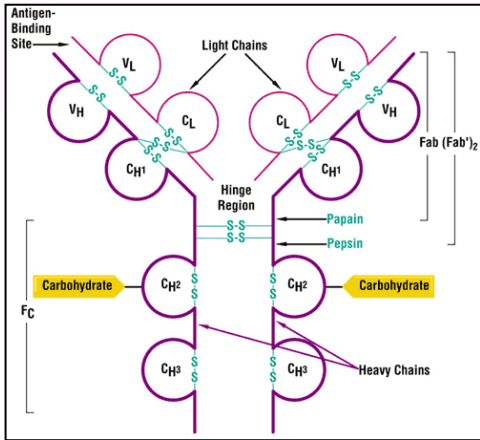
6300 POTENTIAL RECOMBINATIONS

185+165 POTENTIAL RECOMBINATIONS

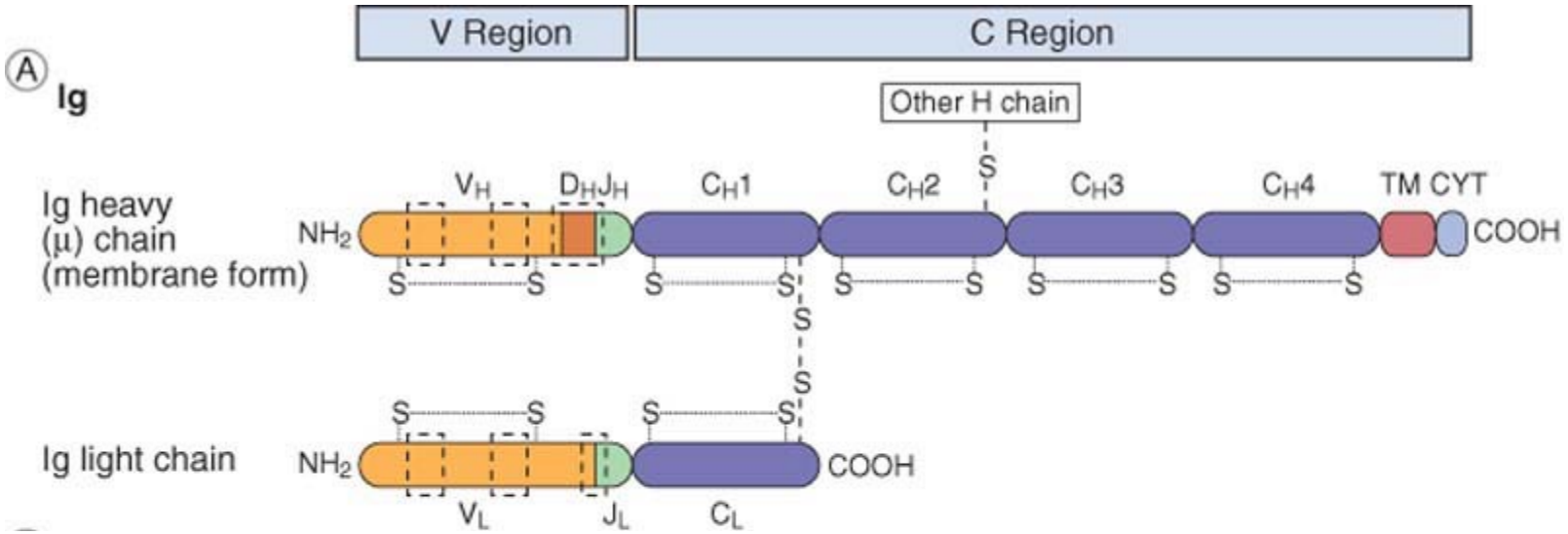
Exonuclease activity
Nucleotide insertion
Somatic hypermutation

6.3×10^6 x1000 3.5×10^5

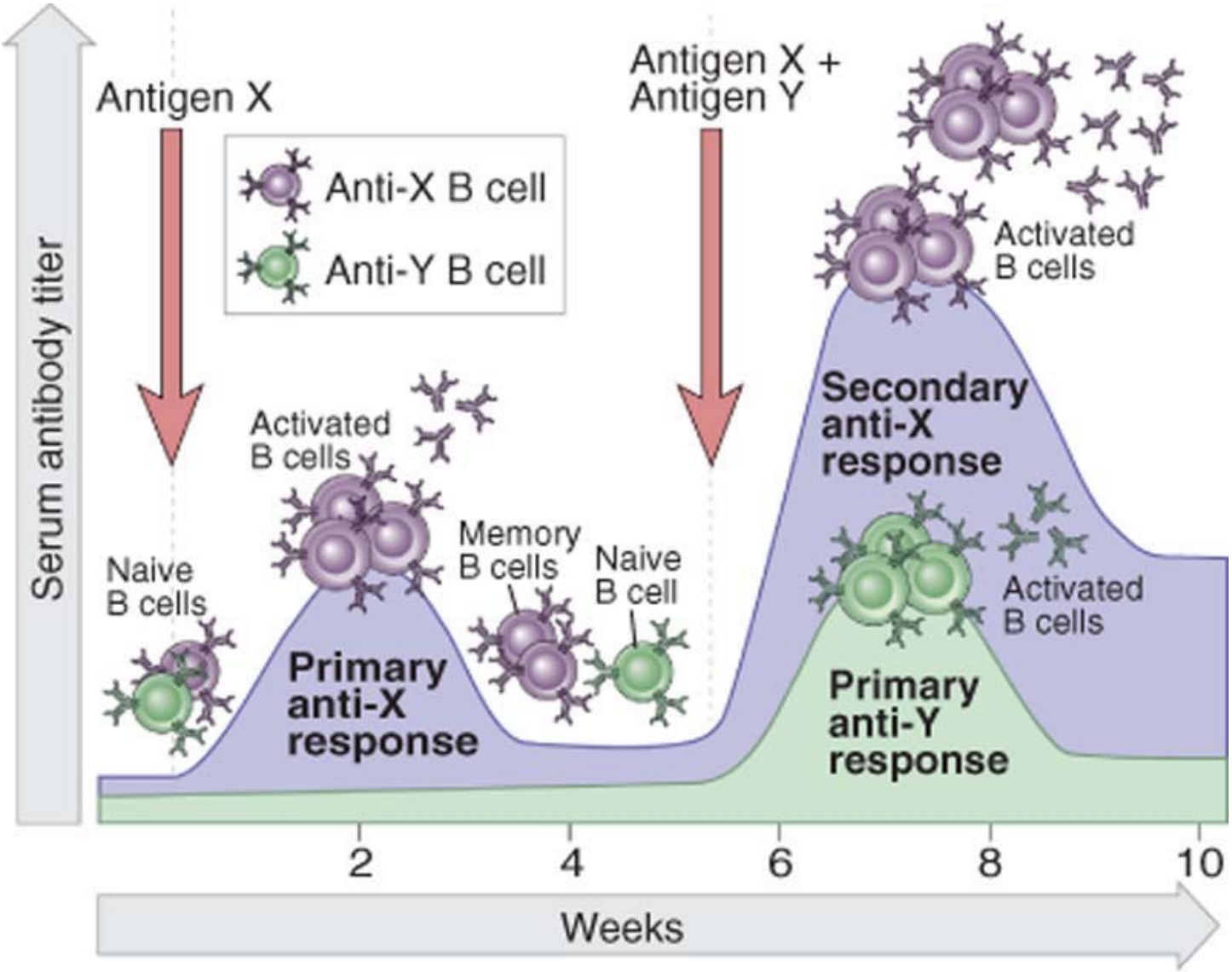
10^{12}



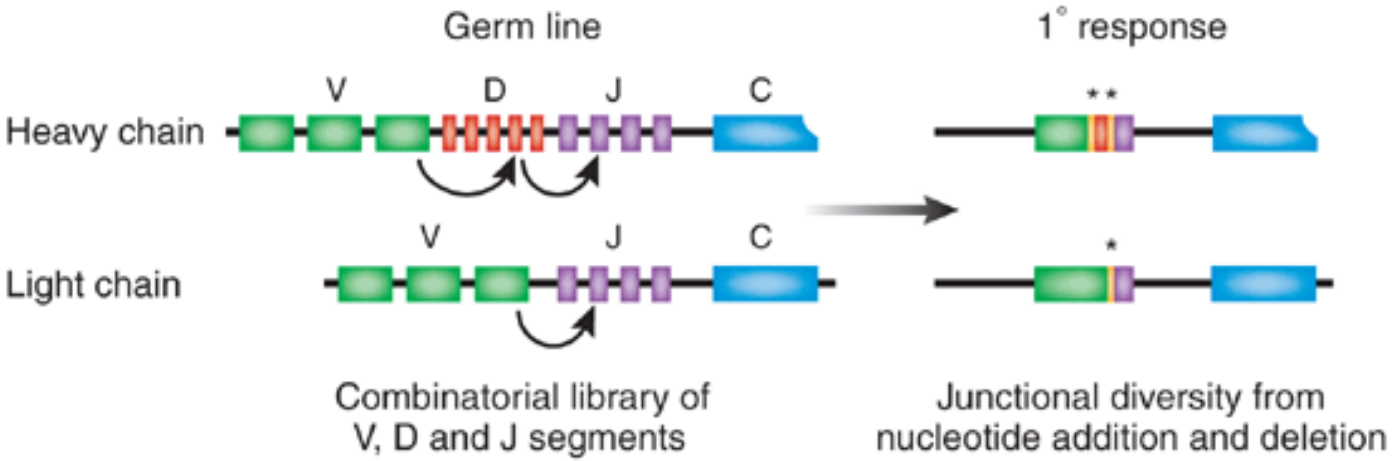
IMGT Repertoire, http://imgt.cines.fr


A
Ig
V Region
C Region
Other H chain
Ig heavy
(μ) chain
(membrane form)
NH2
VH
DHJH
CH1
CH2
CH3
CH4
TM CYT
COOH
S
Ig light chain
NH2
COOH
VL
JL
CL

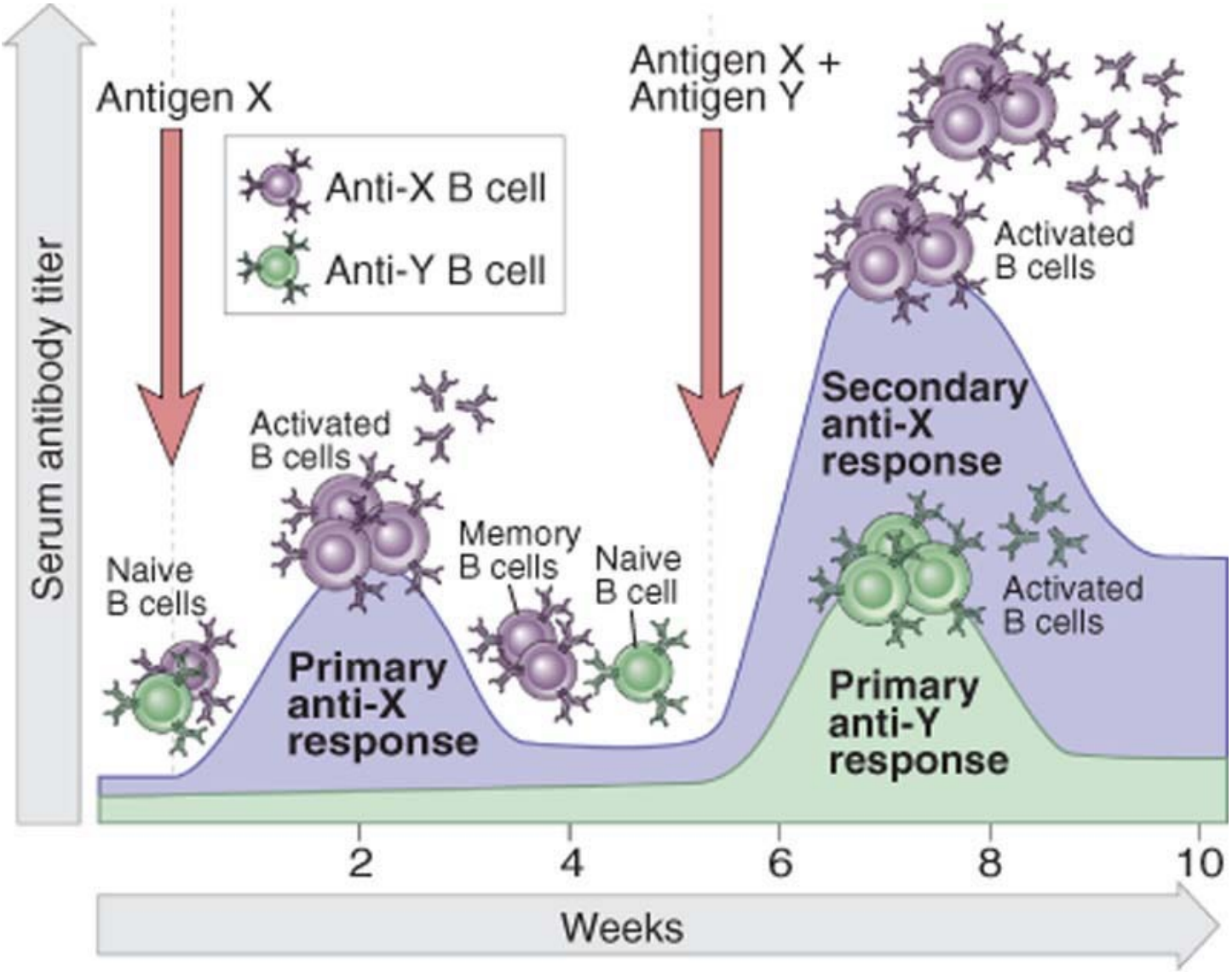
Timing for antibody production



How are antibody genes rearranged in vivo?



Timing for antibody production



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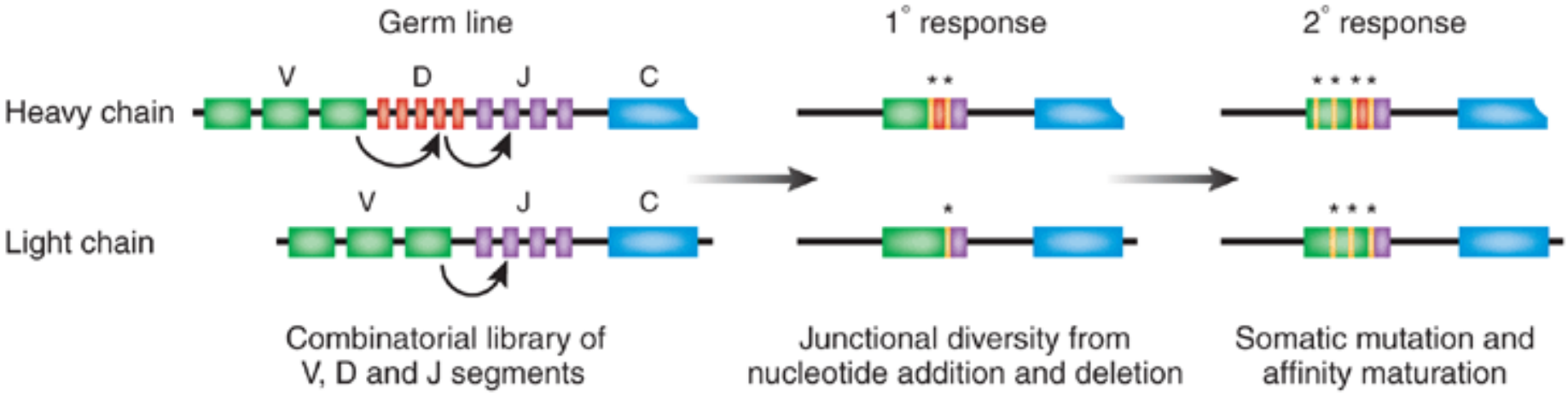
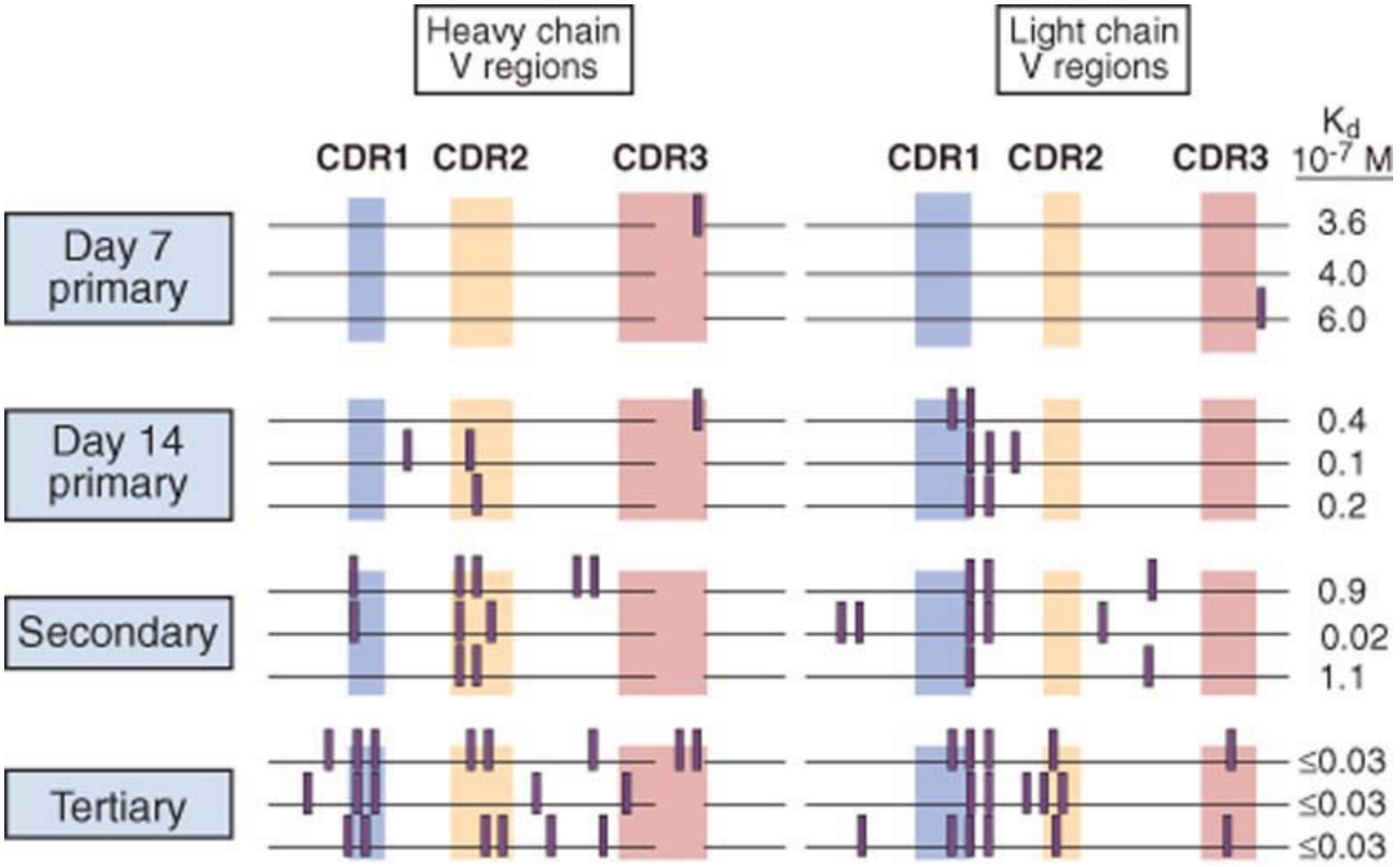
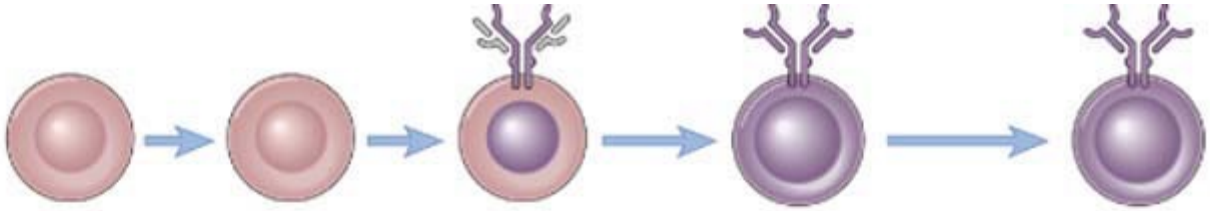




Final variability of antibodies: $>10^{12}$

Where?



Stage of maturation	Stem cell	Pro-B	Pre-B	Immature B	Mature B
Proliferation					
Rag expression					
TdT expression					
Ig DNA, RNA	Unrecombined (germline) DNA	Unrecombined (germline) DNA	Recombined H chain gene (VDJ); μ mRNA	Recombined H chain gene (VDJ), κ or λ genes (VJ); μ or κ or λ mRNA	Alternative splicing of VDJ-C RNA (primary transcript), to form C_μ and C_δ mRNA
Ig expression	None	None	Cytoplasmic μ and pre-B receptor–associated μ	Membrane IgM (μ+ κ or λ light chain)	Membrane IgM and IgD
Surface markers	$CD43^+$	$CD43^+$ $CD19^+$ $CD10^+$	$B220^{lo}$ $CD43^+$	IgM^{lo} $CD43^-$	IgD^+ IgM^+ $CD23^+$
Anatomic site	Bone marrow			Periphery	
Response to antigen	None	None	None	Negative selection (deletion), receptor editing	Activation (proliferation and differentiation)

Monoclonal antibodies

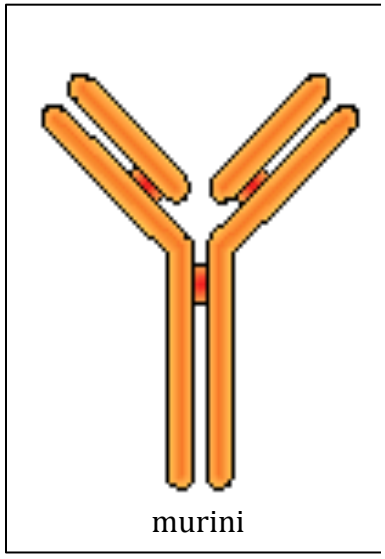
Murine monoclonal antibodies produced by hybridomas

Monoclonal antibodies

Murine monoclonal antibodies produced by hybridomas

Human immune response to mouse antibodies

HAMA = Human Anti-Mouse Antibodies


murini

- Endanger the patient
- enhance the clearance of the Ab
- reduce its therapeutic effect.

Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 6851–6855, November 1984
Immunology

Chimeric human antibody molecules: Mouse antigen-binding domains with human constant region domains

(transfection/protoplast fusion/calcium phosphate transfection/intronic controlling elements/transfectoma)

SHERIE L. MORRISON*, M. JACQUELINE JOHNSON†, LEONARD A. HERZENBERG†, AND VERNON T. OI‡

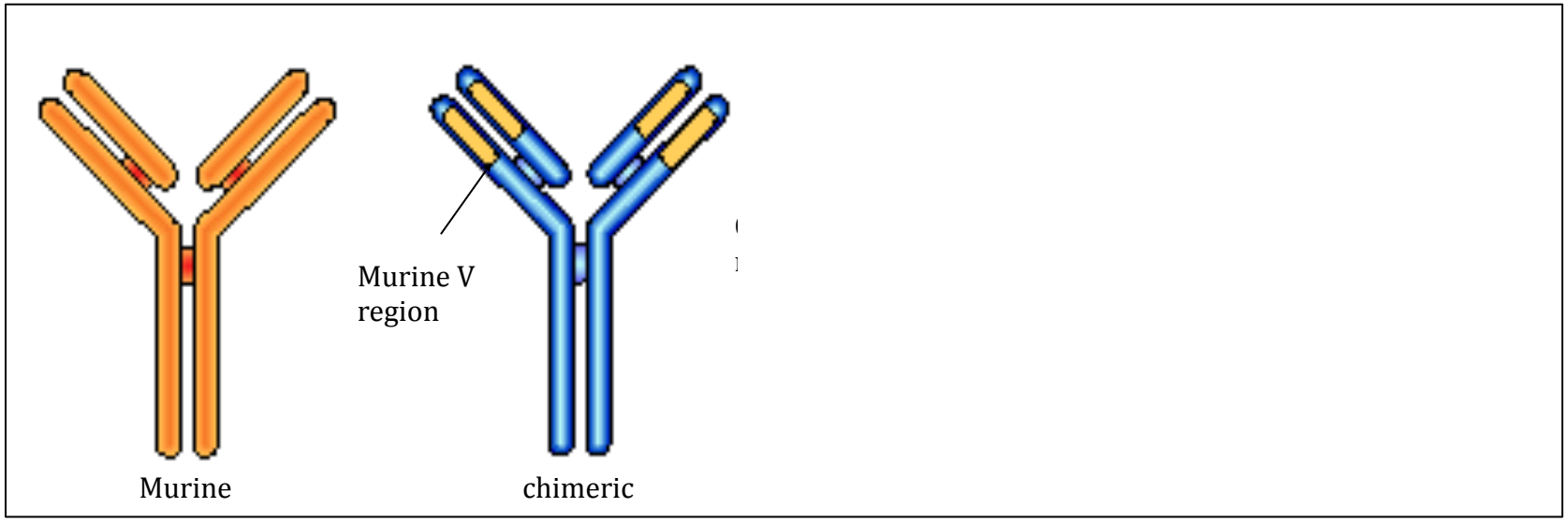
*Department of Microbiology and the Cancer Center, Institute for Cancer Research, College of Physicians and Surgeons, Columbia University, New York, NY 10032; †Department of Genetics, Stanford University School of Medicine, Stanford, CA 94305; and ‡Becton–Dickinson Monoclonal Center, 2375 Garcia Avenue, Mountain View, CA 94043

Contributed by Leonard A. Herzenberg, August 1, 1984

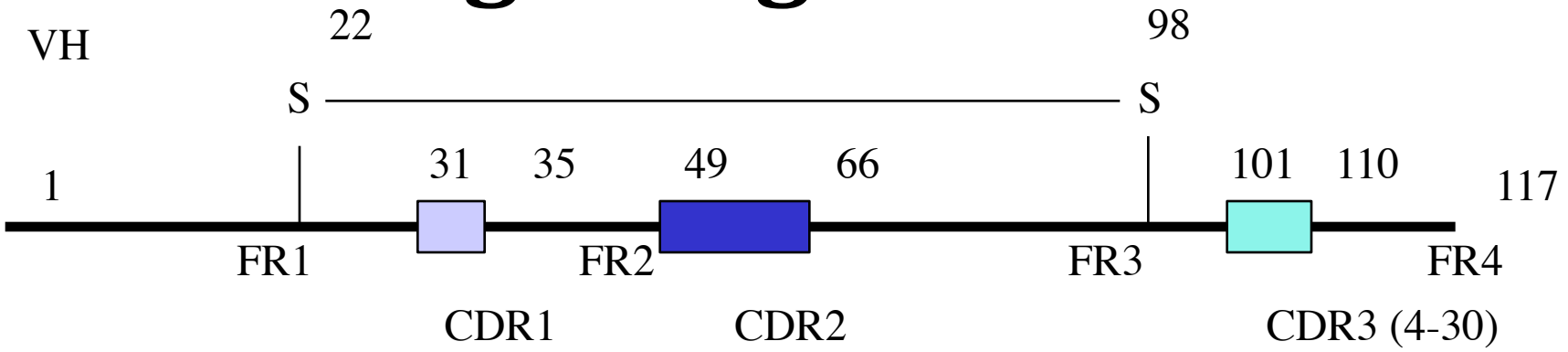
Monoclonal antibodies

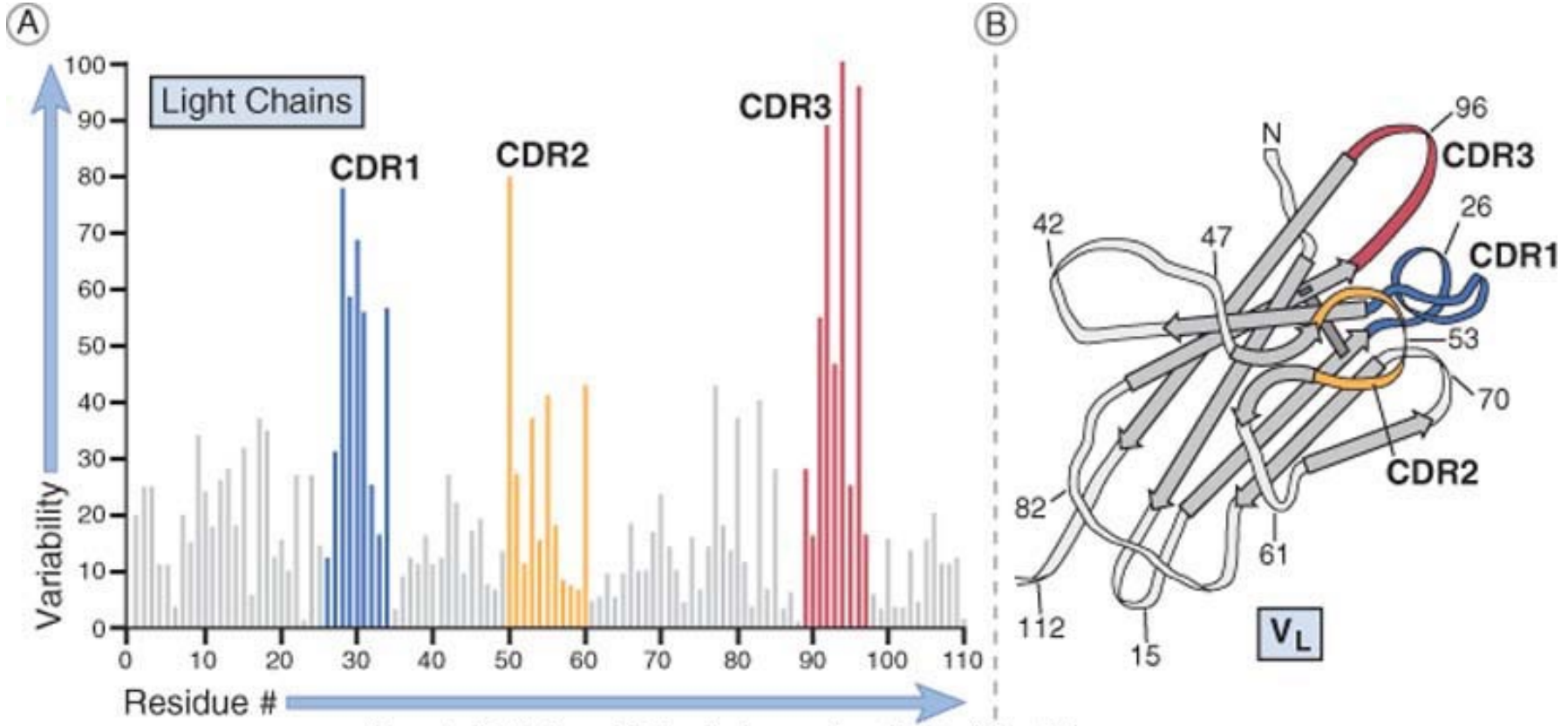
Murine monoclonal antibodies produced by hybridomas

Chimeric recombinant antibodies: 70% human DNA



Rearranged V gene structure





Abbas et al: Cellular and Molecular Immunology, Updated 6th Edition.

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Display Settings: Abstract | Send to:

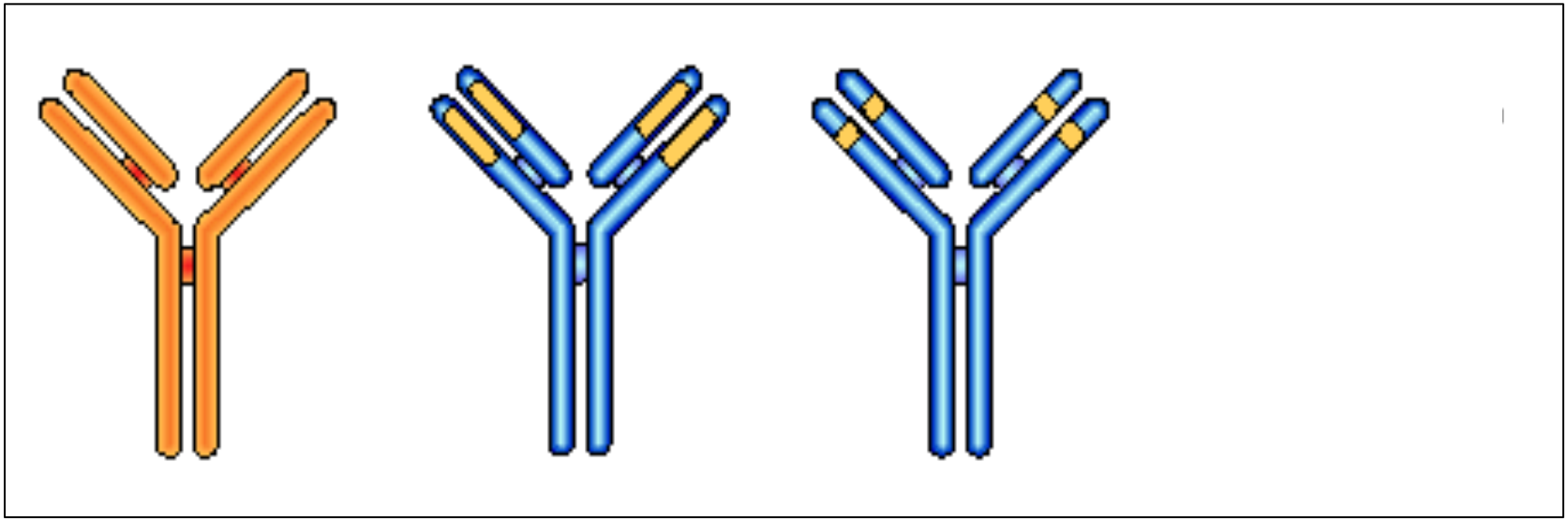
Nature. 1986 May 29-Jun 4;321(6069):522-5.

Replacing the complementarity-determining regions in a human antibody with those from a mouse.

Jones PT, Dear PH, Foote J, Neuberger MS, Winter G.

Abstract

The variable domains of an antibody consist of a beta-sheet framework with hypervariable regions (or complementarity-determining regions--CDRs) which fashion the antigen-binding site. Here we attempted to determine whether the antigen-binding site could be transplanted from one framework to another by grafting the CDRs. We substituted the CDRs from the heavy-chain variable region of mouse antibody B1-8, which binds the hapten NP-cap (4-hydroxy-3-nitrophenacetyl caproic acid; KNP-cap = 1.2 microM), for the corresponding CDRs of a human myeloma protein. We report that in combination with the B1-8 mouse light chain, the new antibody has acquired the hapten affinity of the B1-8 antibody (KNP-cap = 1.9 microM). Such 'CDR replacement' may offer a means of constructing human monoclonal antibodies from the corresponding mouse monoclonal antibodies.



- Humanized antibodies (95% human DNA umano; only CDRs are murine)

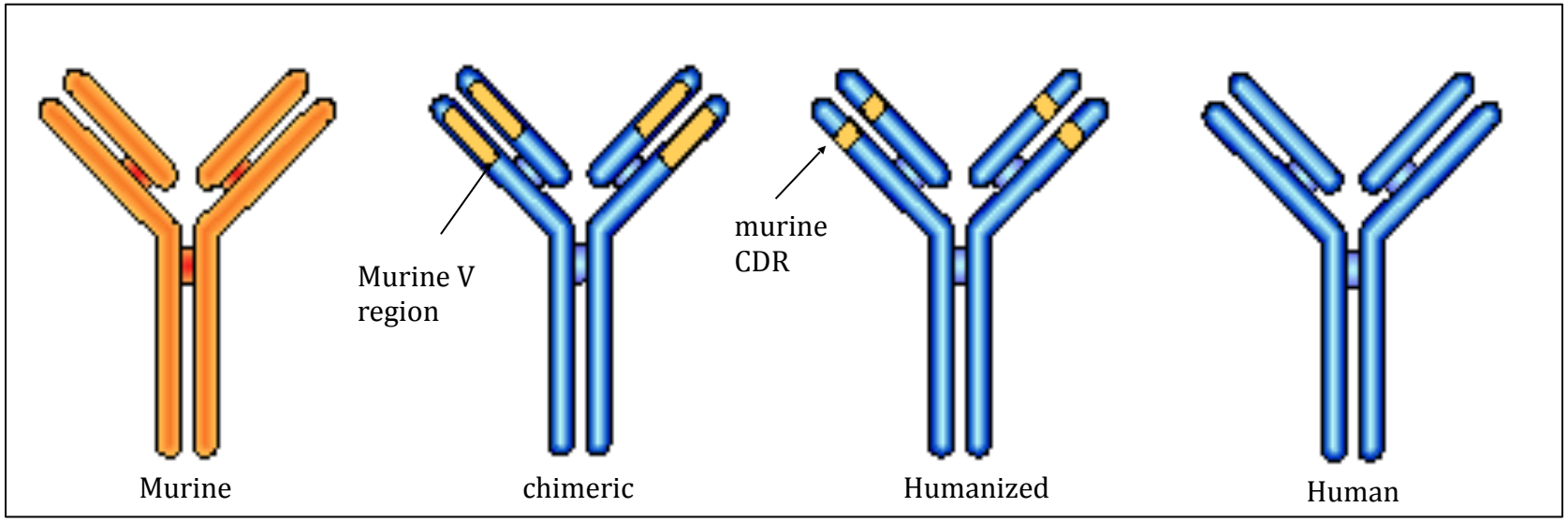
Monoclonal antibodies

Murine monoclonal antibodies produced by hybridomas

Chimeric recombinant antibodies: 70% human DNA

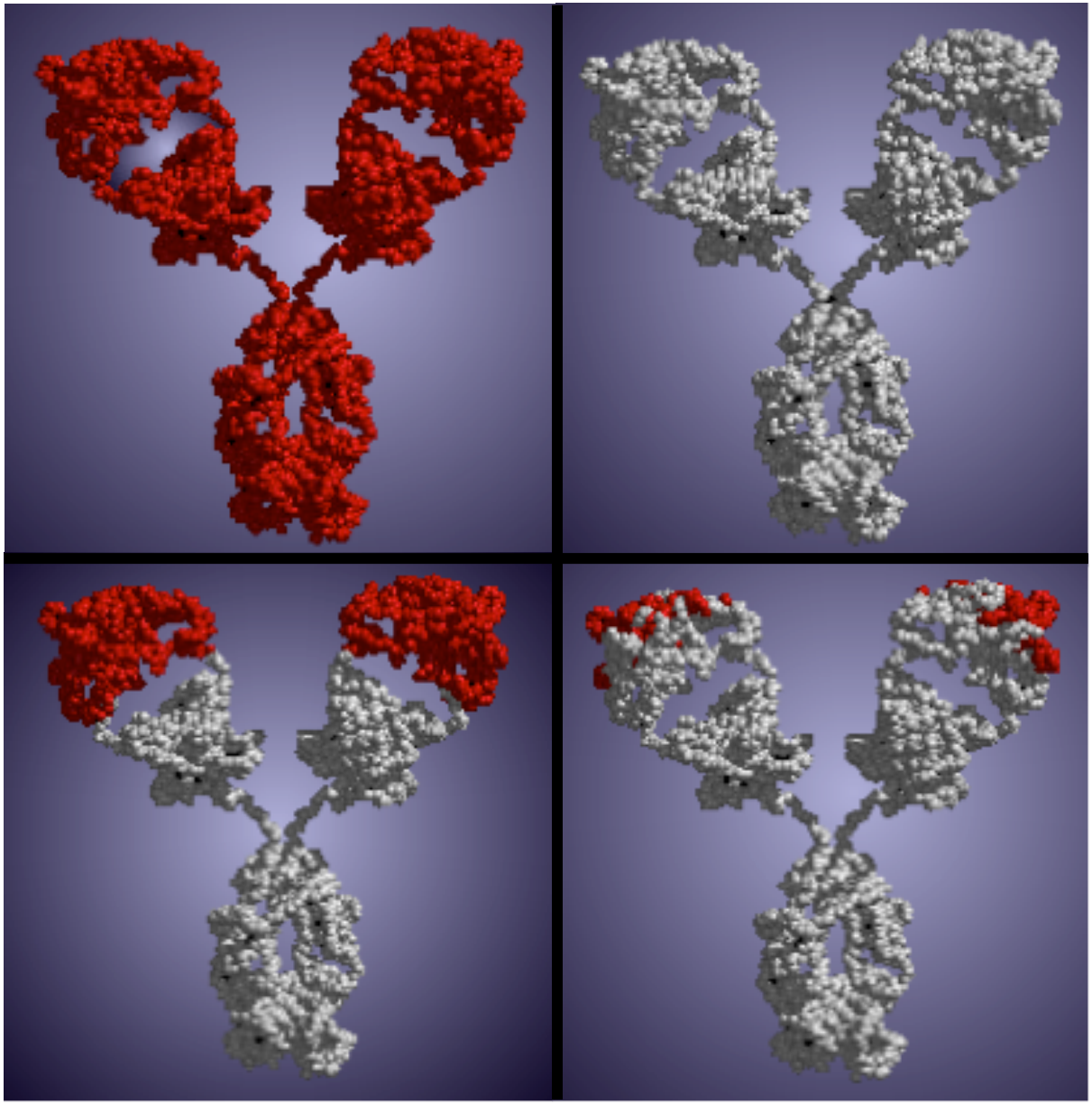
Humanized recombinant antibodies: 95% human DNA

Human antibodies: 100% human DNA



What they really look like

Mouse



Human

Chimeric

Humanized

Rapid identification of a human antibody with high prophylactic and therapeutic efficacy in three animal models of SARS-CoV-2 infection

Wei Li[a,1,2], Chuan Chen[a,1], Aleksandra Drelich[b,1], David R. Martinez[c,1], Lisa E. Gralinski[c,1], Zehua Sun[a,1], Alexandra Schäfer[c,1], Swarali S. Kulkarni[d,1], Xianglei Liu[a], Sarah R. Leist[c], Doncho V. Zhelev[a], Liyong Zhang[a], Ye-Jin Kim[a], Eric C. Peterson[e], Alex Conard[e], John W. Mellors[a,e], Chien-Te K. Tseng[b], Darryl Falzarano[d], Ralph S. Baric[c], and Dimiter S. Dimitrov[a,e,2]

[a]Department of Medicine, Division of Infectious Diseases, Center for Antibody Therapeutics, University of Pittsburgh Medical School, Pittsburgh, PA 15261; [b]Department of Microbiology and Immunology, Centers for Biodefense and Emerging Diseases, Galveston National Laboratory, Galveston, TX 77550; [c]Department of Epidemiology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599; [d]Department of Veterinary Microbiology, Vaccine and Infectious Disease Organization–International Vaccine Centre, University of Saskatchewan, Saskatoon, SK S7N 5E3, Canada; and [e]Abound Bio, Pittsburgh, PA 15219

Edited by Adolfo Garcia-Sastre, Icahn School of Medicine at Mount Sinai, New York, NY, and approved September 30, 2020 (received for review May 20, 2020)

Effective therapies are urgently needed for the SARS-CoV-2/COVID-19 pandemic. We identified panels of fully human monoclonal antibodies (mAbs) from large phage-displayed Fab, scFv, and VH libraries by panning against the receptor binding domain (RBD) of the SARS-CoV-2 spike (S) glycoprotein. A high-affinity Fab was selected from one of the libraries and converted to a full-size antibody, IgG1 ab1, which competed with human ACE2 for binding to RBD. It potently neutralized replication-competent SARS-CoV-2 but not SARS-CoV, as measured by two different tissue culture assays, as well as a replication-competent mouse ACE2-adapted SARS-CoV-2 in BALB/c mice and native virus in hACE2-expressing transgenic mice showing activity at the lowest tested dose of 2 mg/kg. IgG1 ab1 also exhibited high prophylactic and therapeutic efficacy in a hamster model of SARS-CoV-2 infection. The mechanism of neutralization is by competition with ACE2 but could involve antibody-dependent cellular cytotoxicity (ADCC) as IgG1 ab1 had ADCC activity in vitro. The ab1 sequence has a relatively low number of somatic mutations, indicating that ab1-like antibodies could be quickly elicited during natural SARS-CoV-2 infection or by RBD-based vaccines. IgG1 ab1 did not aggregate, did not exhibit other developability liabilities, and did not bind to any of the 5,300 human membrane-associated proteins tested. These results suggest that IgG1 ab1 has potential for therapy and prophylaxis of SARS-CoV-2 infections. The rapid identification (within 6 d of availability of antigen for panning) of potent mAbs shows the value of large antibody libraries for response to public health threats from emerging microbes.

therapeutic antibodies | coronaviruses | SARS-CoV-2 | animal models

The severe acute respiratory distress syndrome coronavirus 2 (SARS-CoV-2) (1) has spread worldwide thus requiring safe additional affinity maturation. Our exceptionally potent antibody against the MERS-CoV, m336, was directly selected from a very large (size ~10^{11} clones) library from 50 individuals (4). However, another potent antibody, m102.4, against henipaviruses was additionally affinity matured from its predecessor selected from a smaller library (size ~10^{10} clones) from 10 individuals (6). Thus, to generate high-affinity and safe mAbs we used very large (size ~10^{11} clones each) naive human antibody libraries in Fab, scFv, or VH format using peripheral blood mononuclear cells (PBMCs) from a total of 490 individuals obtained before the SARS-CoV-2 outbreak. The complementarity-determining regions (CDRs) of the human VH domains were grafted (except CDR1 which was mutagenized or grafted) from our other libraries as previously described (12).

Significance

Effective therapies are urgently needed for COVID-19. We rapidly (within a week) identified a fully human monoclonal germline-like antibody (ab1) from phage-displayed libraries that potently inhibited mouse ACE2-adapted SARS-CoV-2 replication in wild-type BALB/c mice and native virus in transgenic mice expressing human ACE2 as well as in hamsters when administered before virus challenge. It was also effective when administered after virus infection of hamsters, although at lower efficacy than when used prophylactically. Ab1 was highly specific and did not bind to human cell membrane-associated proteins. It also exhibited good developability properties including complete lack of aggregation. Ab1 has potential for prophylaxis and therapy of COVID-19 alone or in combination with other agents.

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DOI: 10.1002/ctm2.184

CLINICAL AND TRANSLATIONAL MEDICINE Open Access

WILEY

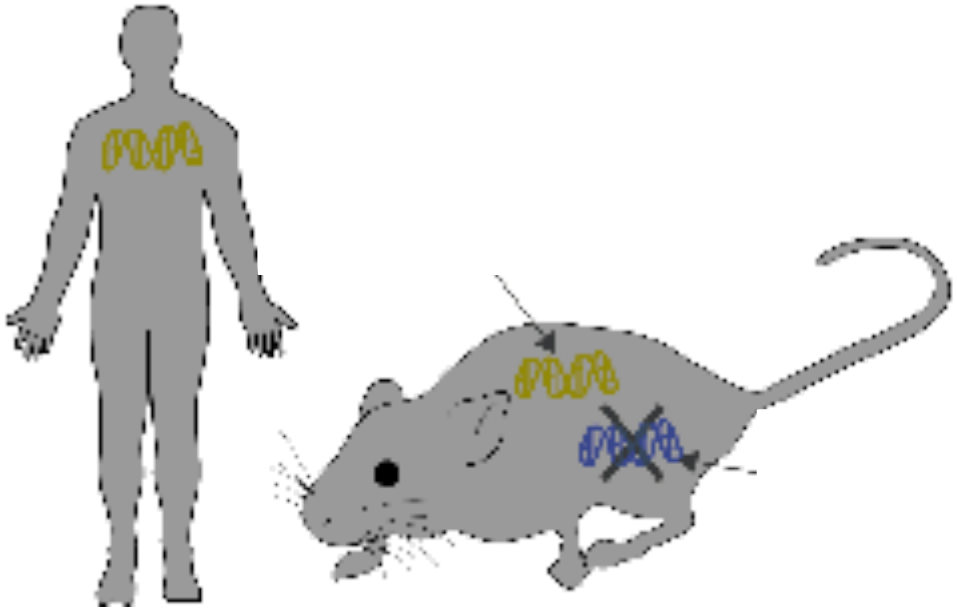
LETTER TO EDITOR

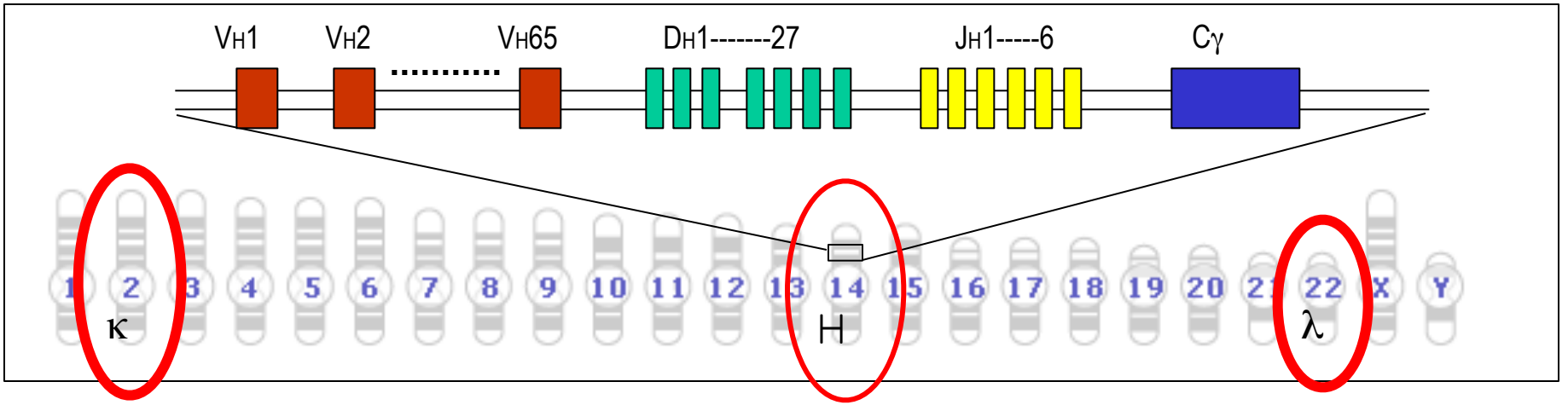
Immunological strategies against spike protein: Neutralizing antibodies and vaccine development for COVID-19

Making human antibodies

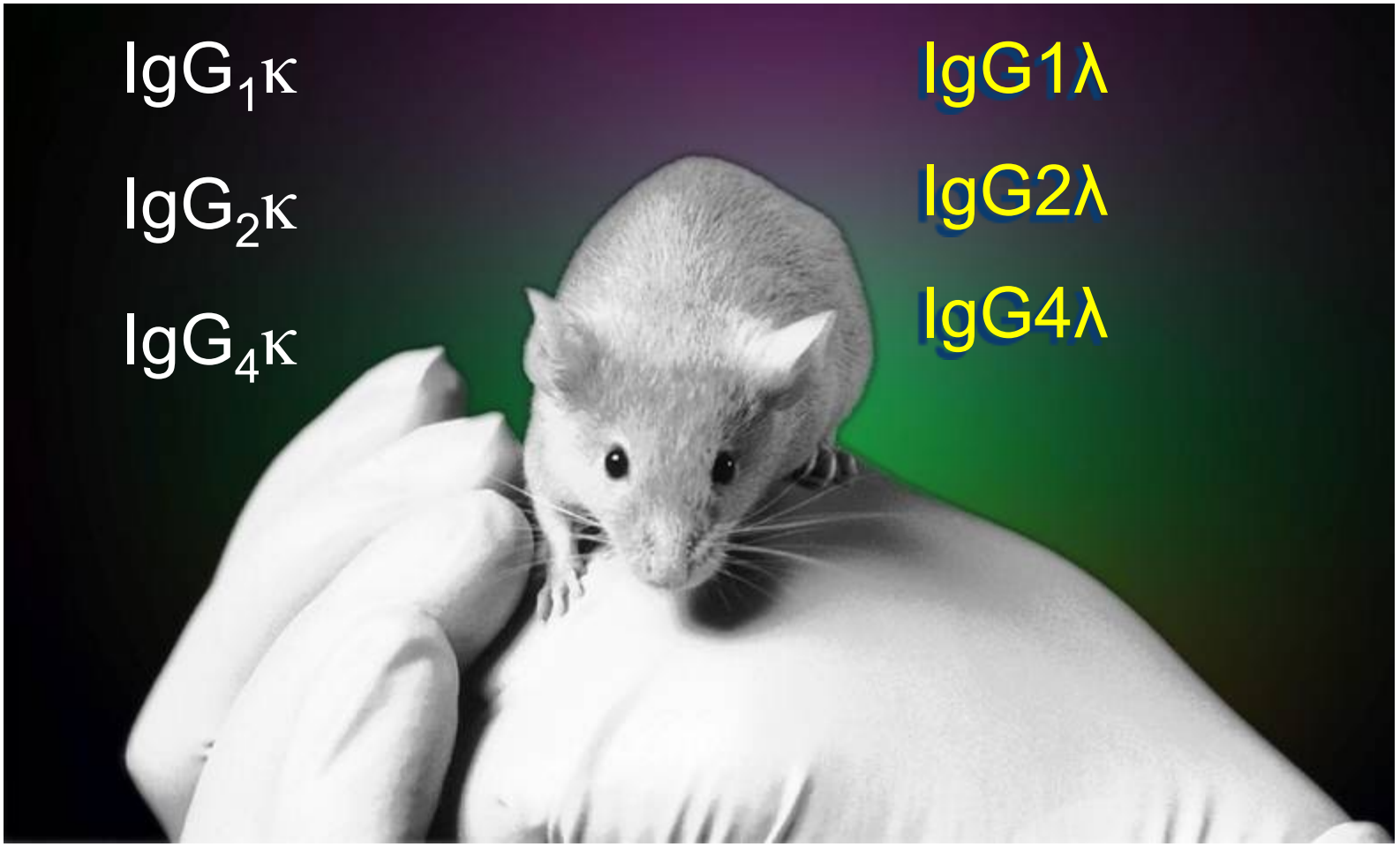
- Transgenic animals
- Phage/microbial/yeast display

Transgenic Mouse





XenoMouse®-κλ Strains



Nature Biotechnology **25**, 1134 - 1143 (2007)
Published online: 5 October 2007 | doi:10.1038/nbt1337

From XenoMouse technology to panitumumab, the first fully human antibody product from transgenic mice

Aya Jakobovits[1], Rafael G Amado[2], Xiaodong Yang[3], Lorin Roskos[4] & Gisela Schwab[5]

Therapeutic monoclonal antibodies have shown limited efficacy and safety owing to immunogenicity of mouse sequences in humans. Among the approaches developed to overcome these hurdles were transgenic mice genetically engineered with a 'humanized' humoral immune system. One such transgenic system, the XenoMouse, has succeeded in recapitulating the human antibody response in mice, by introducing nearly the entire human immunoglobulin loci into the germ line of mice with inactivated mouse antibody machinery. XenoMouse strains have been used to generate numerous high-affinity, fully human antibodies to targets in multiple disease indications, many of which are progressing in clinical development. However, validation of the technology has awaited the recent regulatory approval of panitumumab (Vectibix), a fully human antibody directed against epidermal growth factor receptor (EGFR), as treatment for people with advanced colorectal cancer. The successful development of panitumumab represents a milestone for mice engineered with a human humoral immune system and their future applications.

Immunological Reviews

Ian N. Foltz
Kannan Gunasekaran
Chadwick T. King

Discovery and bio-optimization of human antibody therapeutics using the XenoMouse® transgenic mouse platform

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This article is part of a series of reviews covering Immunoglobulins: from genes to therapies appearing in Volume 270 of Immunological Reviews.

Immunological Reviews 2016
Vol. 270: 51–64

Immunological Reviews
0105-2896

Summary: Since the late 1990s, the use of transgenic animal platforms has transformed the discovery of fully human therapeutic monoclonal antibodies. The first approved therapy derived from a transgenic platform – the epidermal growth factor receptor antagonist panitumumab to treat advanced colorectal cancer – was developed using XenoMouse® technology. Since its approval in 2006, the science of discovering and developing therapeutic monoclonal antibodies derived from the XenoMouse® platform has advanced considerably. The emerging array of antibody therapeutics developed using transgenic technologies is expected to include antibodies and antibody fragments with novel mechanisms of action and extreme potencies. In addition to these impressive functional properties, these antibodies will be designed to have superior biophysical properties that enable highly efficient large-scale manufacturing methods. Achieving these new heights in antibody drug discovery will ultimately bring better medicines to patients. Here, we review best practices for the discovery and bio-optimization of monoclonal antibodies that fit functional design goals and meet high manufacturing standards.

Keywords: antibody therapeutics, XenoMouse®, drug discovery, monoclonal antibodies, bio-optimization

Introduction and overview of XenoMouse®

The field of therapeutic monoclonal antibodies was revolutionized in the 1990s with the development of transgenic animal systems capable of producing high-quality human antibody repertoires. The original transgenic mouse platforms capable of producing human antibodies were the XenoMouse®, developed by the biotechnology company Cell-Genesys (later Abgenix Inc., and now part of Amgen Inc., Thousand Oaks, CA, USA), and the HuMAb-Mouse®, developed by Genpharm Inc. (later Medarex Inc., now part of Bristol-Myers Squibb Inc., New York, NY, USA). This invention has had a significant effect on human health, with more than 10 human antibody therapeutics derived from transgenic animals currently approved for human use (1). The technology used to develop these transgenic platforms

Transgenic mice

- These produce human antibodies following immunization
- The creation of monoclonal antibodies is the same as normal mice using hybridoma technology
- Antibody production is still required after selection

Making human antibodies

- Transgenic animals
- Phage/microbial/yeast display

The Royal Swedish Academy of Sciences has decided to award the **Nobel Prize in Chemistry 2018** with one half to Frances H. Arnold "for the directed evolution of enzymes" and the other half **jointly to George P. Smith and Sir Gregory P. Winter "for the phage display of peptides and antibodies**."

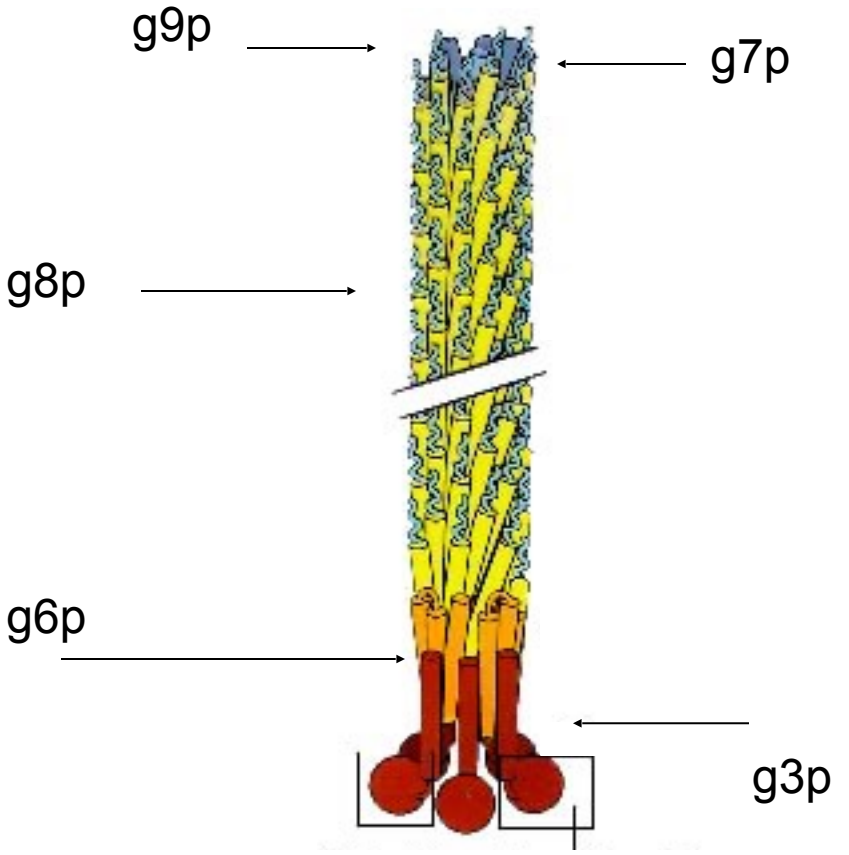
The power of evolution is revealed through the diversity of life. **The 2018 Nobel Laureates in Chemistry have taken control of evolution** and used it for purposes that bring the greatest benefit to humankind. Enzymes produced through directed evolution are used to manufacture everything from biofuels to pharmaceuticals. Antibodies evolved using a method called phage display can combat autoimmune diseases and, in some cases, cure metastatic cancer.

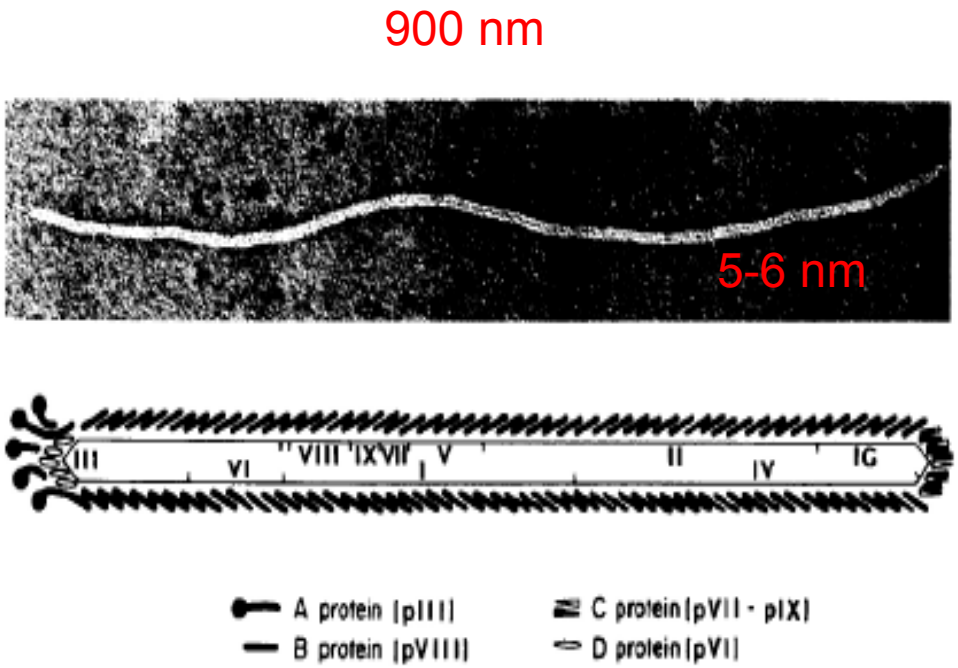
This year's Nobel Laureates have been inspired by the power of evolution and used the same principles – genetic change and selection – to develop proteins that solve humankind's chemical problems.

Antibody libraries

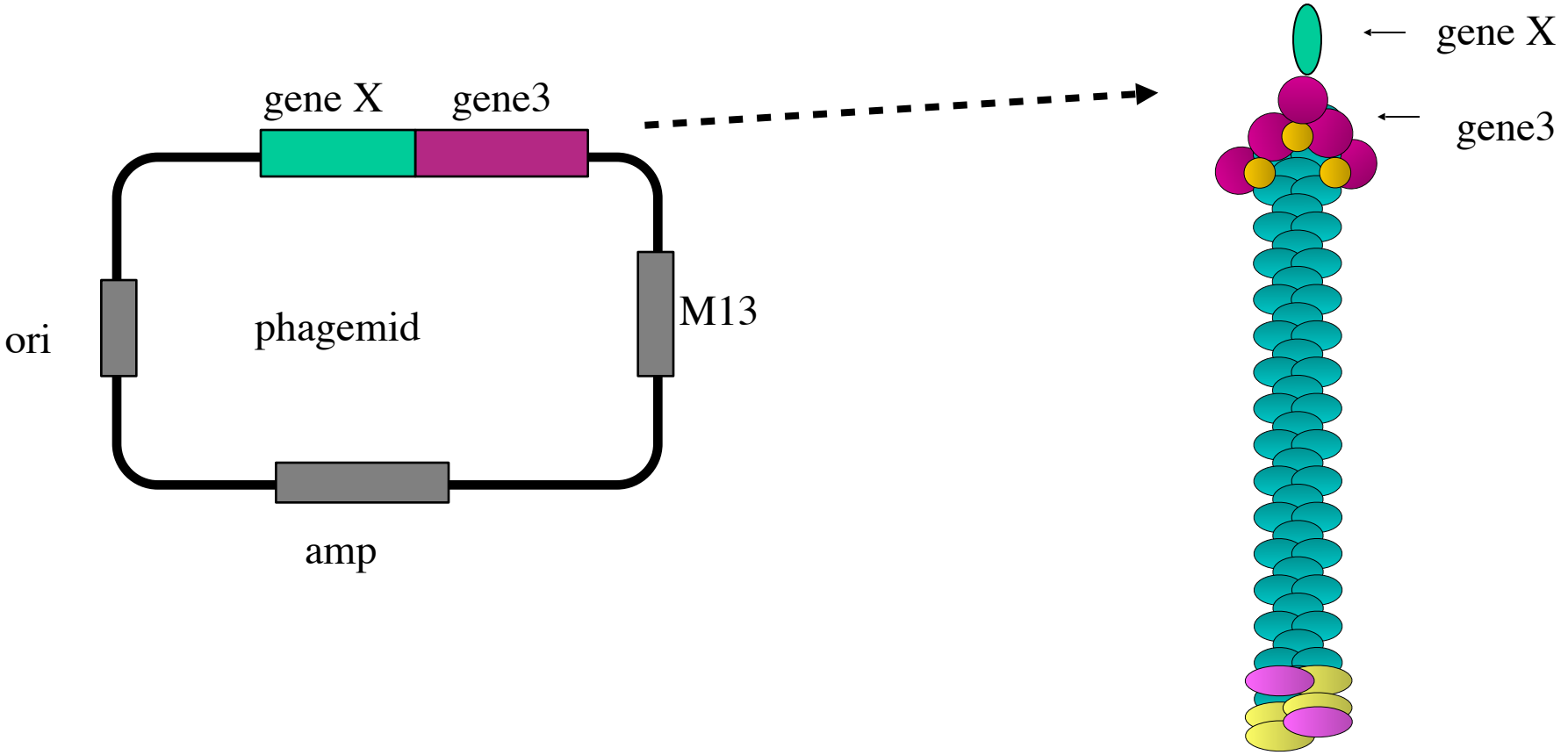
AIM: to allow the possibility to work with the antibody variability in the lab and not in the animal house

The biology of filamentous phages (f1)

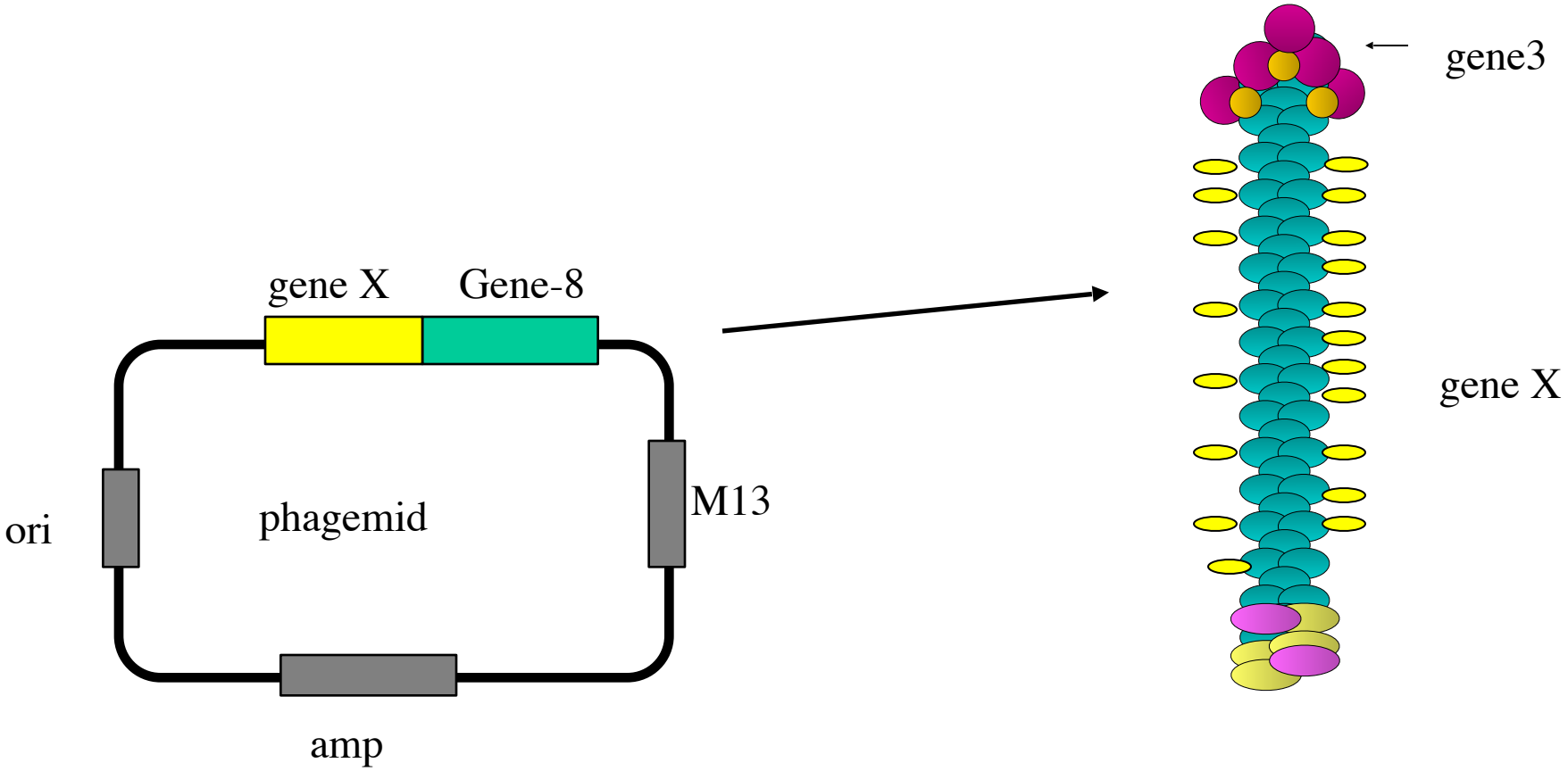




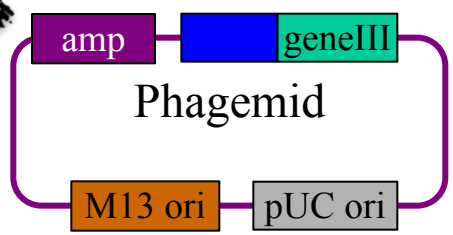
Phage Display Vector

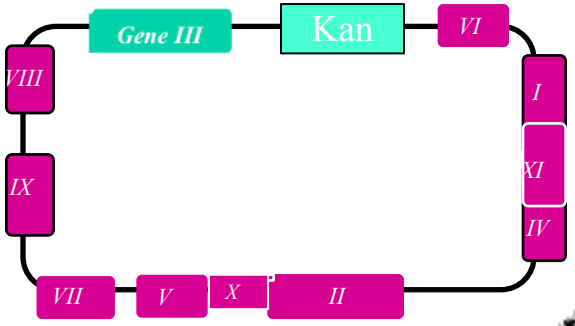


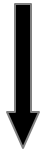
Phage Display Vector



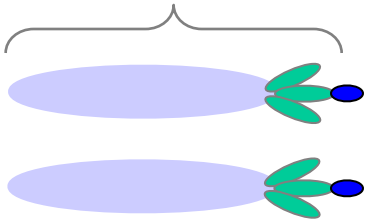
Phage production

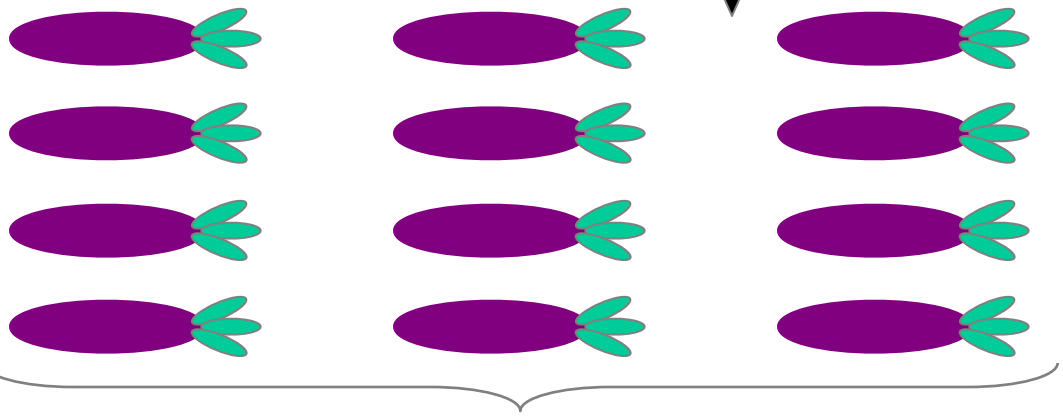




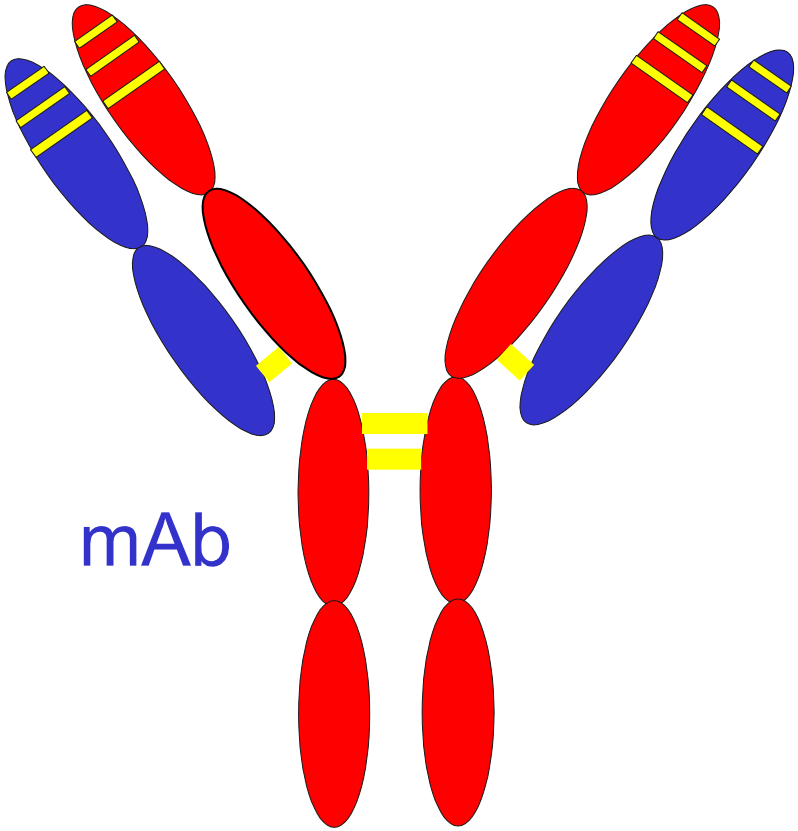


Infective (2. kan)

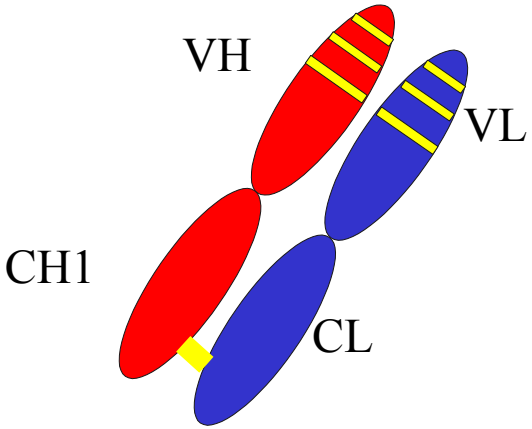




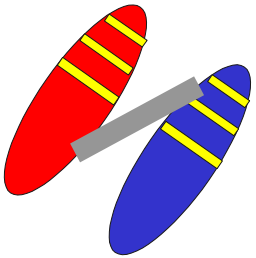
Infective (1. amp)



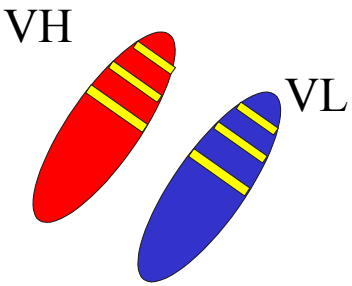
mAb



FAb



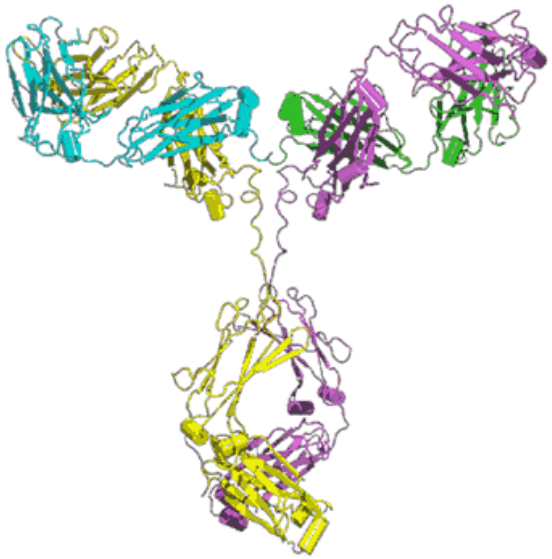
scFv



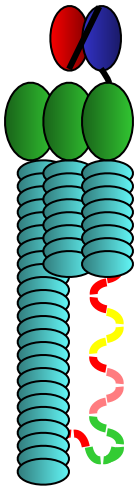
Fv

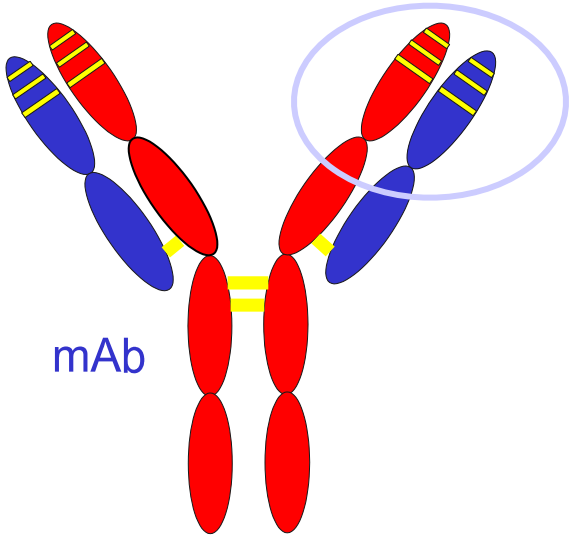
Single chain Fv fragment

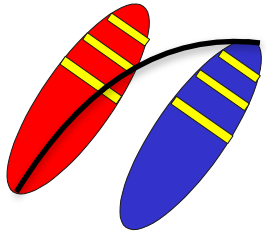
Antibody



Phage



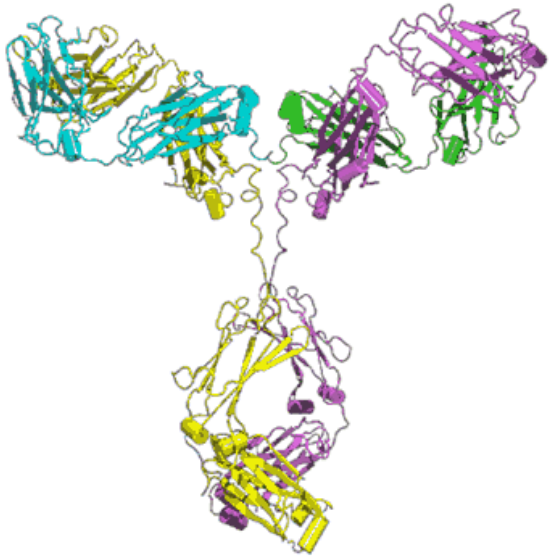


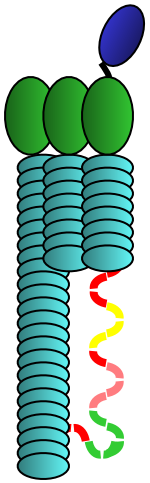


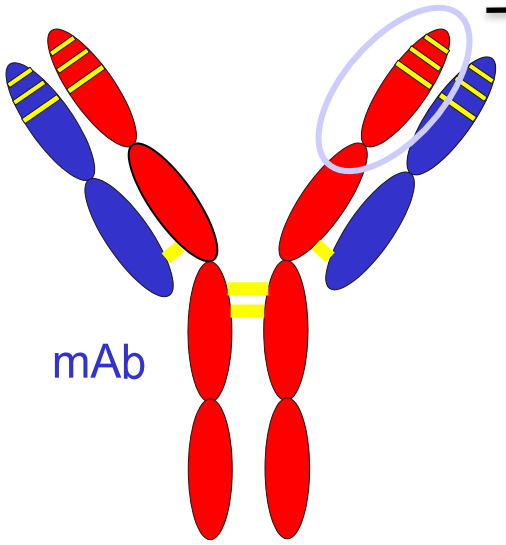
scFv

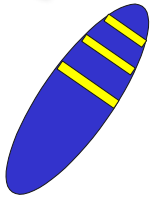
Antibody

Phage



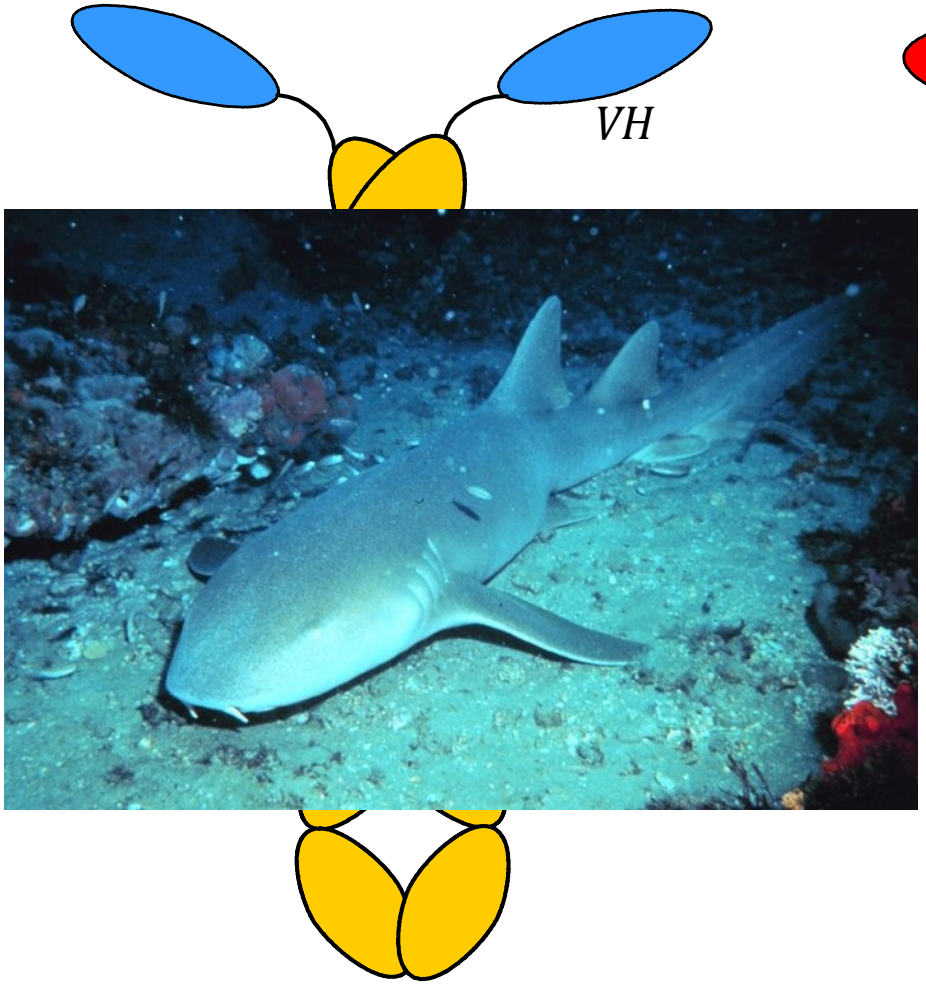






dAb

Some animals use natural dAbs



Shark

Camel lama

Points to consider when making and using antibody libraries

- The source of V region diversity (natural or synthetic)
- For natural V genes, the tissue source of V genes

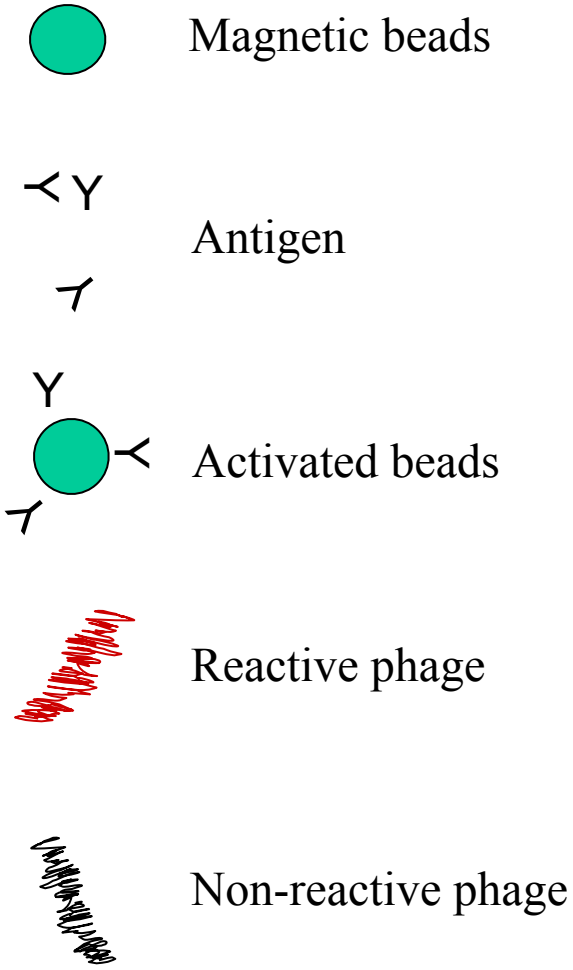
V gene sources for making antibody libraries

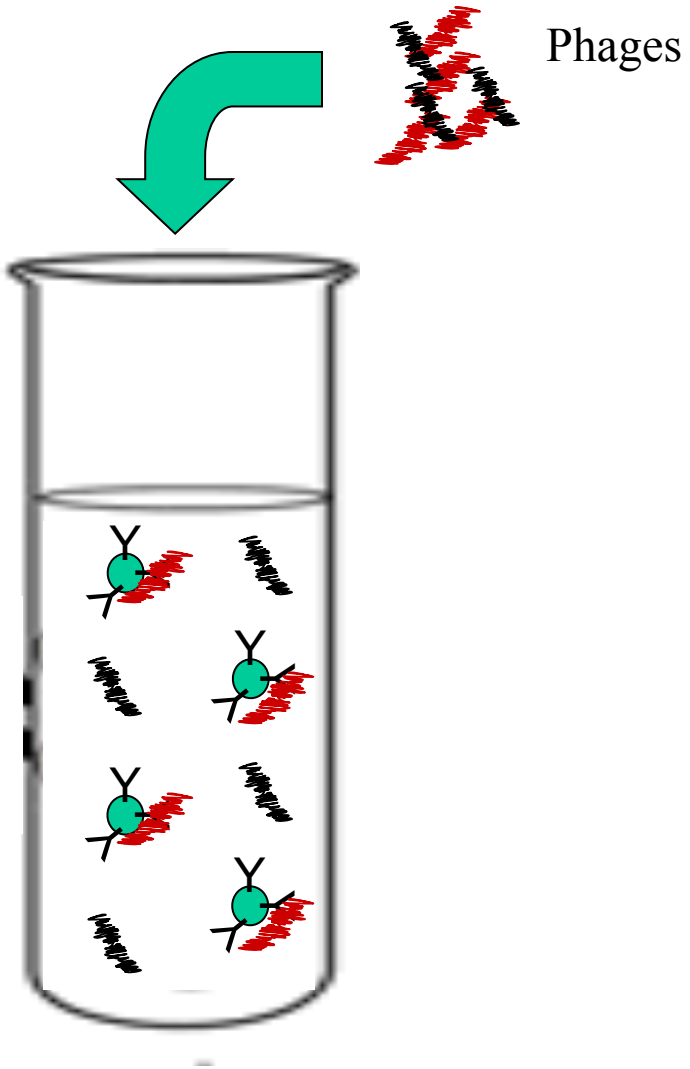
- **Immune sources**: IgG V genes derived from PBL, or spleen from immunized animals or patients
  - High affinity antibodies against single antigens
- **Naive sources**: IgM/IgG V genes, derived from PBL, spleen, bone marrow
  - Antibodies against a wide diversity of antigens
- **Synthetic**: clone all or some V genes (human or mouse) and reconstitute CDRs for both VH and VL by PCR using oligonucleotides

Points to consider when making and using antibody libraries

- Antibody form (Single chain Fv (scFv) or Fab)
- The source of V region diversity (natural or synthetic)
- For natural V genes, the tissue source of V genes
- How to assemble the V regions
- Type of library
- The display protein used (p3, p8 or p9)
- **Selection strategy**

Selection


Magnetic beads
Antigen
Activated beads
Reactive phage
Non-reactive phage


Phages

Selection

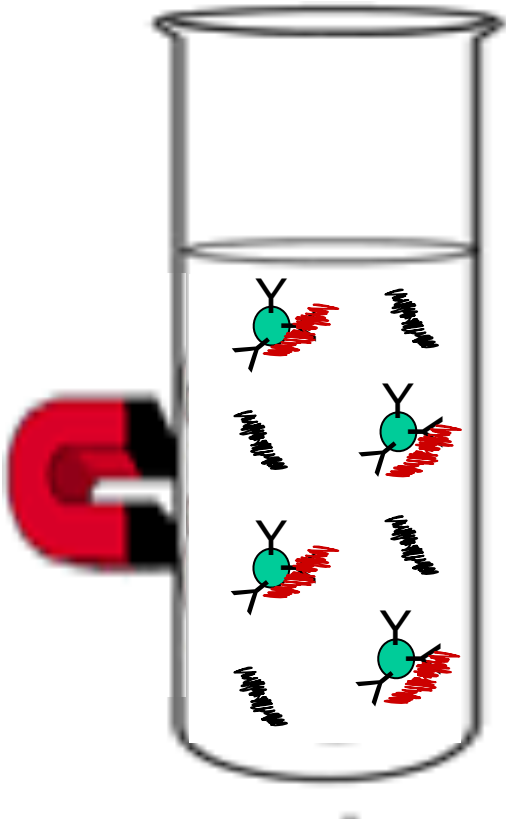
Magnetic beads

Antigen

Activated beads

Reactive phage

Non-reactive phage



Selection

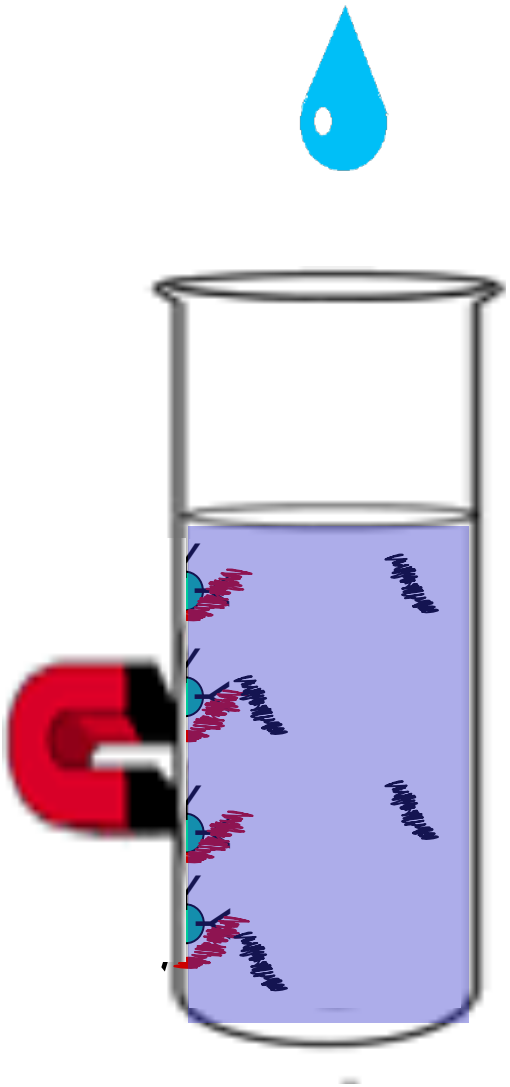
Sfere paramagnetiche

Antigene

Sfere attivate

Reactive phage

Non-reactive phage



Selezione

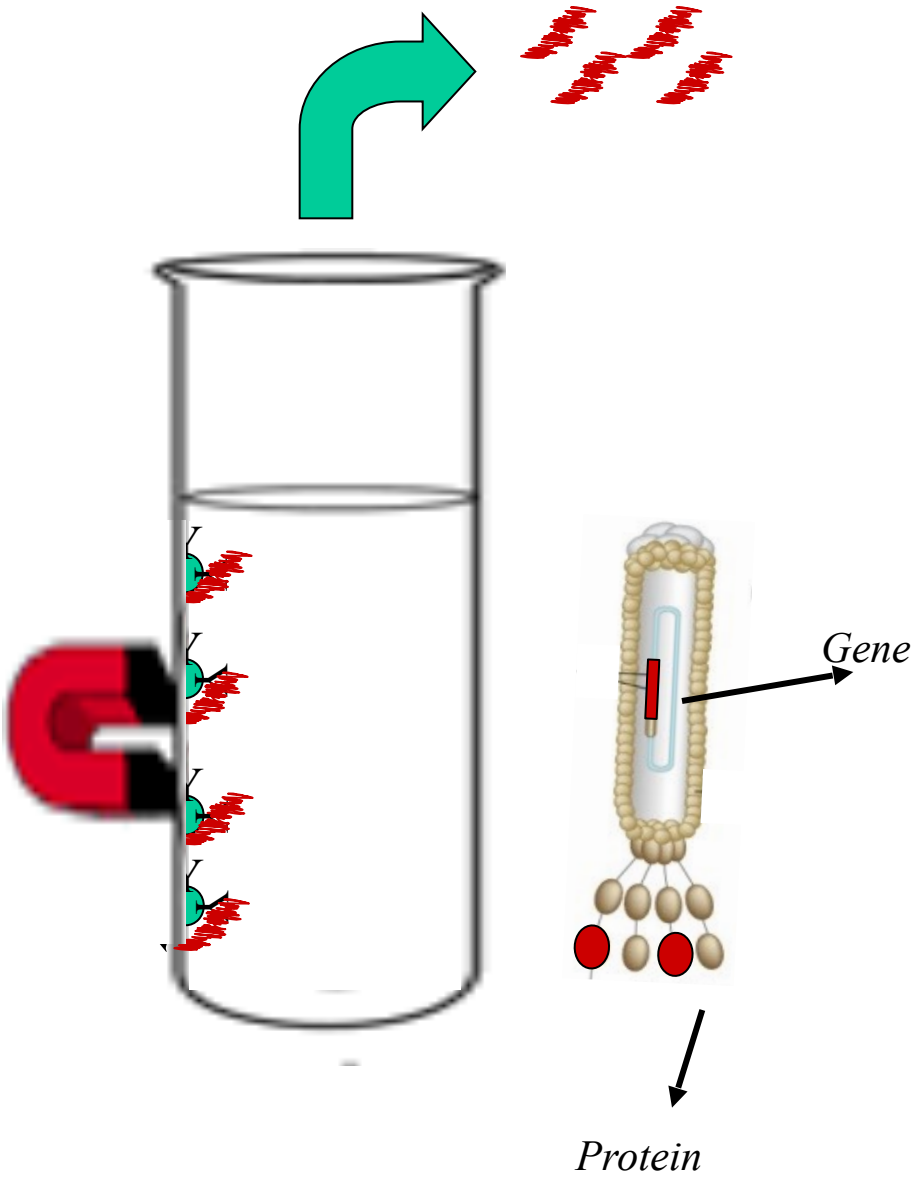
Magnetic beads

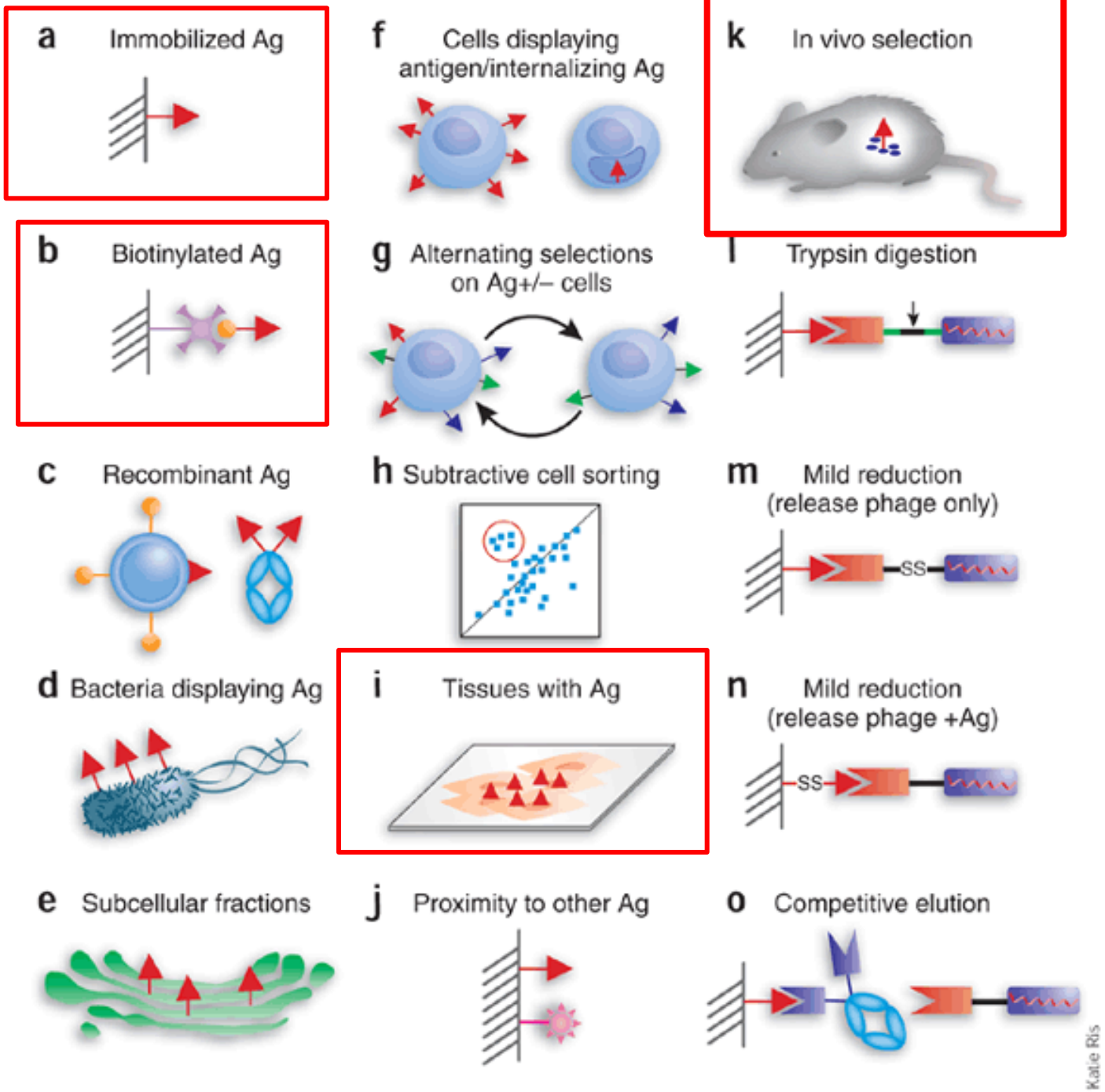
Antigen

Activated beads

Reactive phage

Non-reactive phage




a Immobilized Ag
b Biotinylated Ag
c Recombinant Ag
d Bacteria displaying Ag
e Subcellular fractions
f Cells displaying antigen/internalizing Ag
g Alternating selections on Ag+/– cells
h Subtractive cell sorting
i Tissues with Ag
j Proximity to other Ag
k In vivo selection
l Trypsin digestion
m Mild reduction (release phage only)
SS
n Mild reduction (release phage +Ag)
SS
o Competitive elution
Katie Ris

ARTHRITIS & RHEUMATISM
Vol. 46, No. 8, August 2002, pp 2109–2120
DOI 10.1002/art.10464

Identification of Synovium-Specific Homing Peptides by In Vivo Phage Display Selection

Lewis Lee,[1] Christopher Buckley,[2] Mark C. Blades,[1] Gabriel Panayi,[1] Andrew J. T. George,[3] and Costantino Pitzalis[1]

Objective. **To identify homing peptides specific for human synovium that could be used as targeting devices for delivering therapeutic/diagnostic agents to human joints.**

Methods. **Human synovium and skin were transplanted into SCID mice. A disulfide-constrained 7–amino acid peptide phage display library was injected intravenously into the animals and synovial homing phage recovered from synovial grafts. Following 3–4 cycles of enrichment, DNA sequencing of homing phage clones allowed the identification of specific peptides that were synthesized by a-fluorenylmethyloxycarbonyl chemistry and used in competitive in vivo assays and immunohistochemistry analyses.**

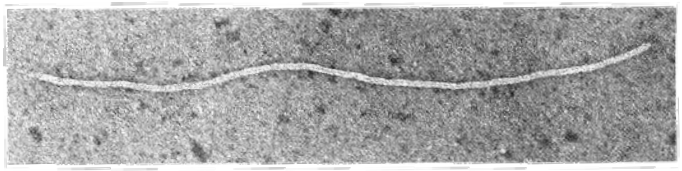
Results. **We isolated synovial homing phages displaying specific peptides that distinctively bound to synovial but not skin or mouse microvascular endothelium (MVE). They retained their tissue homing specificity in vivo, independently from the phage component, the original pathology of the transplanted tissue, and the degree of human/murine graft vascularization. One such peptide (CKSTHDRLC) maintained synovial homing specificity both when presented by the phage and as a free synthetic peptide. The synthetic peptide also competed with and inhibited in vivo the binding of the parent phage to the cognate synovial MVE ligand.**

Conclusion. **This is the first report describing peptides with homing properties specific for human synovial MVE. This was demonstrated using a novel approach targeting human tissues, transplanted into SCID mice, directly by in vivo phage display selection. The identification of such peptides opens the possibility of using these sequences to construct joint-specific drug delivery systems that may have considerable impact in the treatment of arthritic conditions.**

The microvascular endothelium (MVE) plays a major role in the pathogenesis of rheumatoid arthritis (RA), making it an important therapeutic target. RA is a condition characterized by a proliferative synovitis that is responsible for cartilage and bone damage leading to progressive joint destruction (1,2). Florid sprouting of new blood vessels (neoangiogenesis) is typically seen in the early phases of RA synovitis, suggesting that it is a critical element in this condition (3). In the established chronic phase of the disease, the MVE is also important, since it functions as a conduit for the continuous influx of inflammatory cells from the bloodstream into the

Phage antibodies vs. hybridomas

- Antibodies from any species
- In vitro
- Immune or naive source
- Billions antibodies screened
- Affinities $\leq 10^{-8}$
- Affinities can be improved $\leq 10^{-9}$
- Libraries difficult to make and use
- Conserved antigens possible
- High throughput possible
- Gene cloned with selection
- Unusual selection strategies



- Requires animals
- Immune source needed
- Hundreds antibodies screened
- Affinities $\leq 10^{-10}$
- Cannot improve affinity
- Technology widely available and works well
- Conserved antigens difficult
- High throughput impossible
- Gene must be cloned separately
- Only immunization

Antibodies from library: advantages 1

- **Antibodies against self or very conserved antigens possible** (e.g. thyroglobulin, TNFa, CEA, MUC1, CD4)
- **Impossible antibodies possible** (e.g. BIP)
- Antibodies with **fine discrimination** (e.g. estrogen, estradiol, targets differing by a single amino acid)
- Antibodies with **very broad specificities** (e.g. post-translational modifications independently of sequence context)
- **Unusual selection strategies** - Subtractive antibodies, cell surface antibodies
- **Recombinant clones: gene cloned with selection**
  - Availability of sequence means selected affinity reagents are never lost, but archivable forever
  - With improvement of gene synthesis, web based distribution of clone sequences can be contemplated in the future
- **Easily manipulated for downstream uses**
  - Enzyme fusions, multimerization etc.

Antibodies from library: advantages 2

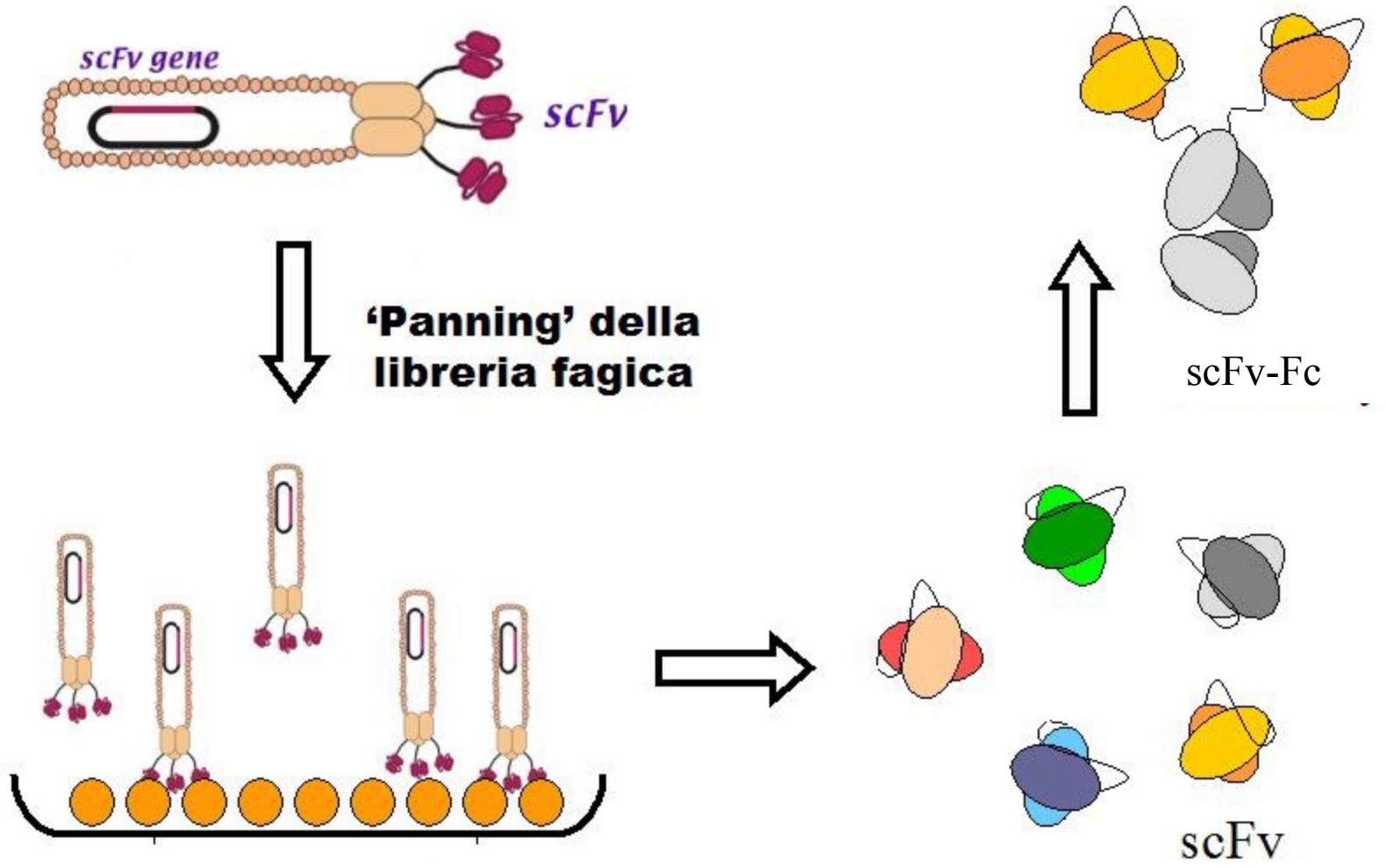
- **Isolate numerous antibodies per antigen in two weeks** (simple selections)
- **Process multiple antigens**: ≥10 manually, 96 well format feasible
- Antibodies isolated can be **high affinity**
- Affinity can be further improved
- Human antibodies can be isolated
- **Affinities 1nM-10μM**
  - Depends upon library quality and degree of effort
  - Can affinity mature to picomolar
- **Truly monoclonal**, make multimeric or polyclonal or oligoclonal as desired
  - Hybridomas often produce multiple different antibodies
  - Less than 33% of commercial antibodies recognize their targets (M. Uhlen - protein atlas)
- **Can be converted back to IgG of any species**

Antibodies from library: disadvantages

- Obtaining or making a library
- Classical monoclonal antibodies work well
- Technique can be difficult to use
- ScFv are produced in bacteria after selection
  - Yields 100μg-10mg/litre
  - scFvs tend to have low stability / storage capability
- Affinities 1nM-10μM
  - Depends upon library quality and degree of effort
- Antibody fragments are monomeric

Points to consider when making and using phage based antibody libraries

- Antibody form (Single chain Fv (scFv) or Fab)
- The source of V region diversity (natural or synthetic)
- For natural V genes, the tissue source of V genes
- How to assemble the V regions
- The display protein used (p3, p8 or p9)
- Phage or phagemid
- Selection strategy
- **Screening**

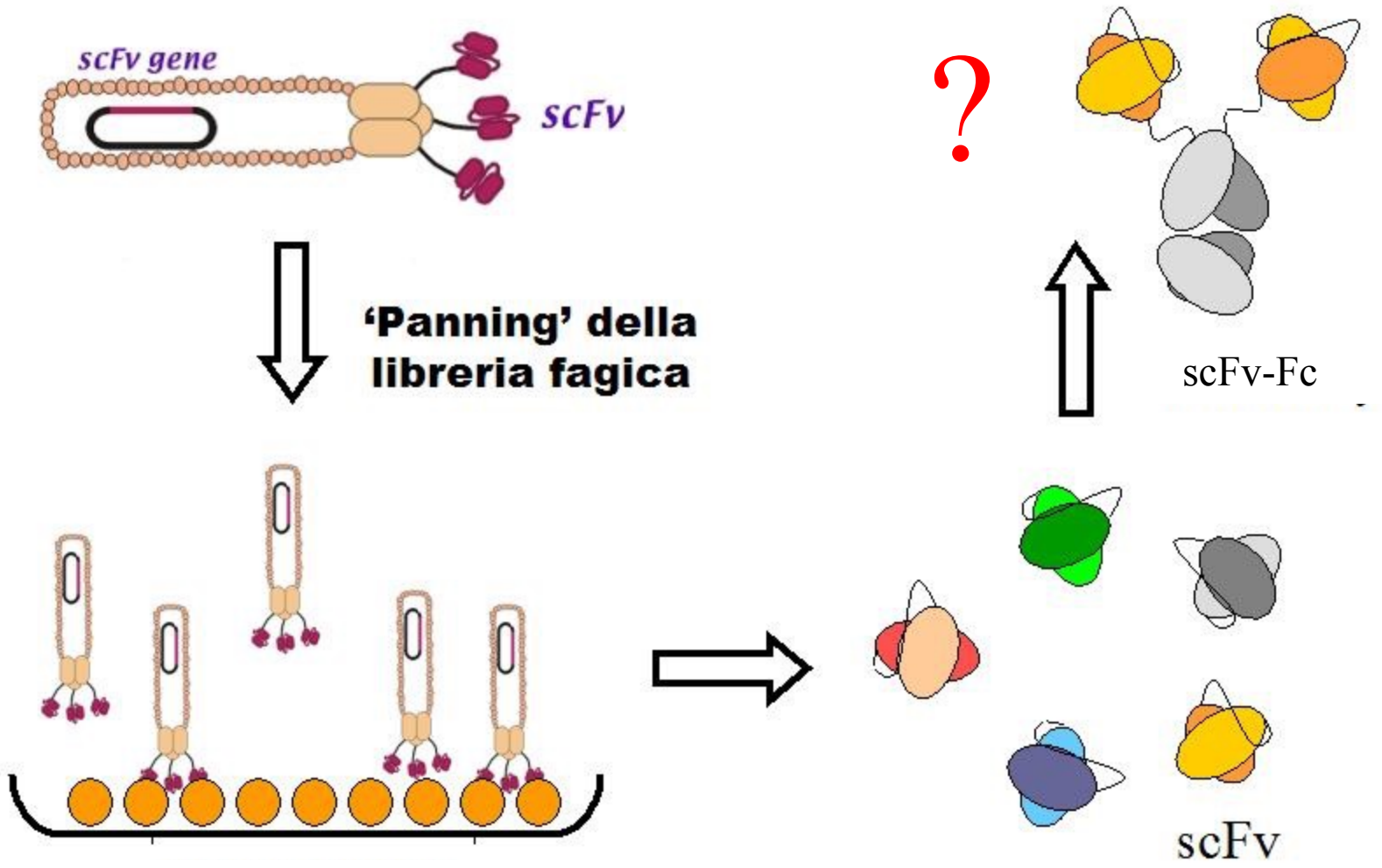

scFv gene
scFv
'Panning' della libreria fagica
scFv-Fc
scFv
?

Antibody screening: tests

- ELISA (Using supernatant/extract)
- BIACORE (affinity using purified molecules)

- Western blot (or immunoistochemistry, or….)

- Functional screening


scFv gene
scFv
'Panning' della libreria fagica
?
scFv-Fc
scFv

Antibody modifications

- Increase affinity
  - Chain shuffling
  - Point mutation

- Modification to Fc portion
  - Change Fc (IgG, IgM, IgA; mouse, rat, dog, human)

MABS
2016, VOL. 8, NO. 7, 1177–1194
http://dx.doi.org/10.1080/19420862.2016.1212149

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REVIEW

OPEN ACCESS

Phage display-derived human antibodies in clinical development and therapy

André Frenzel[a,b], Thomas Schirrmann[a], and Michael Hust[b]

[a]YUMAB GmbH, Rebenring, Braunschweig; [b]Technische Universität Braunschweig, Institut für Biochemie, Biotechnologie und Bioinformatik, Abteilung Biotechnologie, Braunschweig, Germany

ABSTRACT

Over the last 3 decades, monoclonal antibodies have become the most important class of therapeutic biologicals on the market. Development of therapeutic antibodies was accelerated by recombinant DNA technologies, which allowed the humanization of murine monoclonal antibodies to make them more similar to those of the human body and suitable for a broad range of chronic diseases like cancer and autoimmune diseases. In the early 1990s *in vitro* antibody selection technologies were developed that enabled the discovery of "fully" human antibodies with potentially superior clinical efficacy and lowest immunogenicity.

Antibody phage display is the first and most widely used of the *in vitro* selection technologies. It has proven to be a robust, versatile platform technology for the discovery of human antibodies and a powerful engineering tool to improve antibody properties. As of the beginning of 2016, 6 human antibodies discovered or further developed by phage display were approved for therapy. In 2002, adalimumab (Humira®) became the first phage display-derived antibody granted a marketing approval. Humira® was also the first approved human antibody, and it is currently the best-selling antibody drug on the market. Numerous phage display-derived antibodies are currently under advanced clinical investigation, and, despite the availability of other technologies such as human antibody-producing transgenic mice, phage display has not lost its importance for the discovery and engineering of therapeutic antibodies.

Here, we provide a comprehensive overview about phage display-derived antibodies that are approved for therapy or in clinical development. A selection of these antibodies is described in more detail to demonstrate different aspects of the phage display technology and its development over the last 25 years.

ARTICLE HISTORY
Received 26 May 2016
Revised 8 July 2016
Accepted 8 July 2016

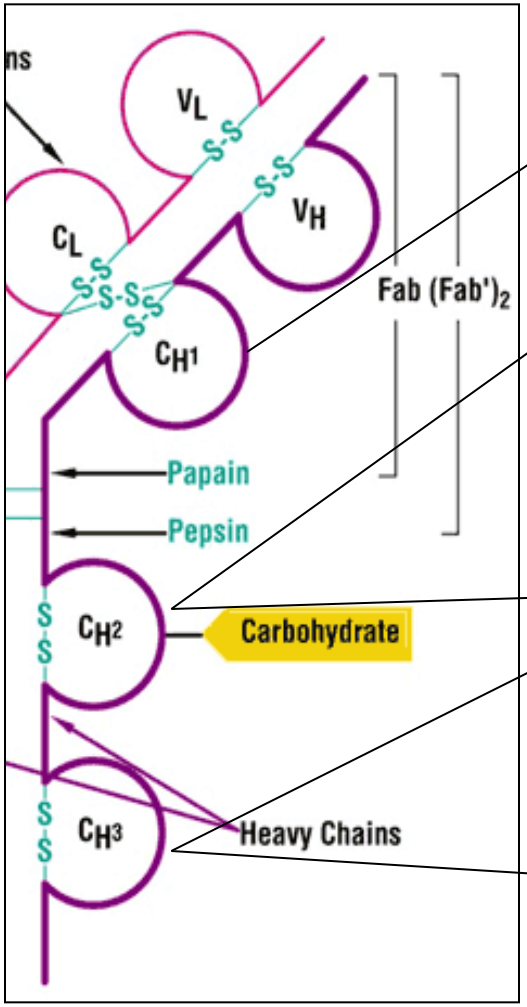
KEYWORDS
Antibody engineering; biologics; clinical development; Fab; human antibodies; phage display; recombinant antibodies; scFv; therapeutic antibodies

Antibody modifications

- Increase affinity
  - Chain shuffling
  - Point mutation

- Modification to Fc portion
  - Change Fc (IgG, IgM, IgA; mouse, rat, human)
  - Enhance CDC
  - Enhance ADCC

Functions of Fc domain



CH1 : - structural function

CH2 : - Complement activation
- ADCC

CH2 - phagocytosis
CH3 - clearance (kidneys)
- placental activity

CH3 : - MAST-cell binding

J Clin Invest. 2015 Oct 5. pii: 82695. doi: 10.1172/JCI82695. [Epub ahead of print]

Sialylation of IgG Fc domain impairs complement-dependent cytotoxicity.

Quast I, Keller CW, Maurer MA, Giddens JP, Tackenberg B, Wang LX, Münz C, Nimmerjahn F, Dalakas MC, Lünemann JD.

Abstract

IgG molecules exert both pro- and antiinflammatory effector functions based on the composition of the fragment crystallizable (Fc) domain glycan. Sialylated IgG Fc domains have antiinflammatory properties that are attributed to their ability to increase the activation threshold of innate effector cells to immune complexes by stimulating the upregulation of the inhibitory Fcγ receptor IIB (FcγRIIB). Here, we report that IgG Fc sialylation of human monoclonal IgG1 molecules impairs their efficacy to induce complement-mediated cytotoxicity (CDC). Fc sialylation of a CD20-targeting antibody had no impact on antibody-dependent cellular cytotoxicity and did not change the affinity of the antibody for activating Fcγ receptors. In contrast, the presence of sialic acid abrogated the increased binding of C1q to Fc-galactosylated IgG1 and resulted in decreased levels of C3b deposition on the cell surface. Similar to monoclonal antibodies, sialic acid inhibited the increased C1q binding to galactosylated Fc fragments in human polyclonal IgG. In sera derived from patients with chronic inflammatory demyelinating polyneuropathy, an autoimmune disease of the peripheral nervous system in which humoral immune responses mediate tissue damage, induction of IgG Fc sialylation was associated with clinical disease remission. Thus, impairment of CDC represents an FcγR-independent mechanism by which Fc-sialylated glycovariants might limit proinflammatory IgG effector functions.

PMID: 26436649 [PubMed - as supplied by publisher]

BioDrugs
https://doi.org/10.1007/s40259-021-00471-0

REVIEW ARTICLE

Recent Achievements and Challenges in Prolonging the Serum Half-Lives of Therapeutic IgG Antibodies Through Fc Engineering

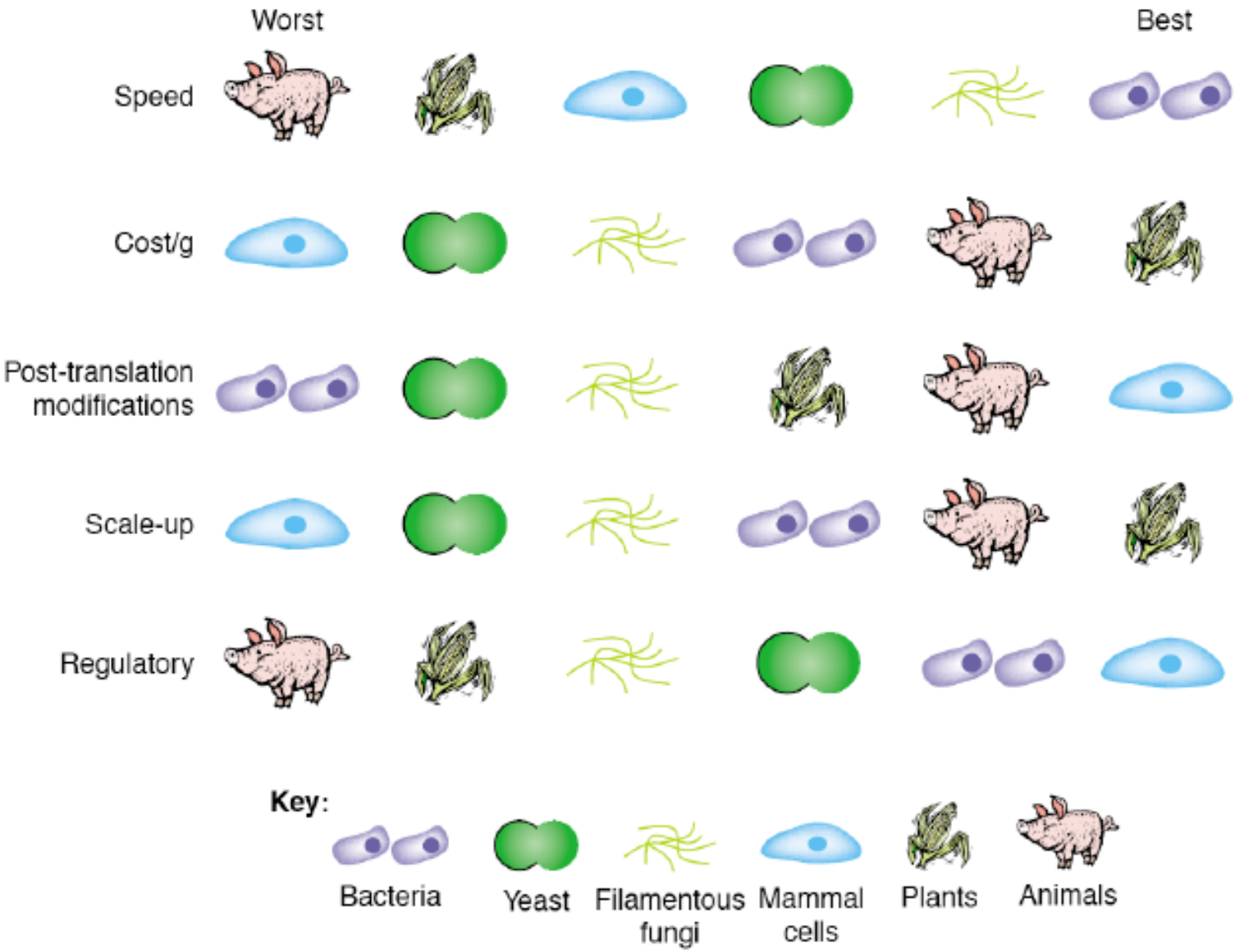
Sanghwan Ko[1,2] · Migyeong Jo[1,3] · Sang Taek Jung[1,2,3,4,5]

Accepted: 7 February 2021

Abstract

Association of FcRn molecules to the Fc region of IgG in acidified endosomes and subsequent dissociation of the interaction in neutral pH serum enables IgG molecules to be recycled for prolonged serum persistence after internalization by endothelial cells, rather than being degraded in the serum and in the lysosomes inside the cells. Exploiting this intracellular trafficking and recycling mechanism, many researchers have engineered the Fc region to further extend the serum half-lives of therapeutic antibodies by optimizing the pH-dependent IgG Fc–FcRn interaction, and have generated various Fc variants exhibiting significantly improved circulating half-lives of therapeutic IgG antibodies. In order to estimate pharmacokinetic profiles of IgG Fc variants in human serum, not only a variety of in vitro techniques to determine the equilibrium binding constants and instantaneous rate constants for pH-dependent FcRn binding, but also diverse in vivo animal models including wild-type mouse, human FcRn transgenic mouse (Tg32 and Tg276), humanized mouse (Scarlet), or cynomolgus monkey have been harnessed. Currently, multiple IgG Fc variants that have been validated for their prolonged therapeutic potency in preclinical models have been successfully entered into human clinical trials for cancer, infectious diseases, and autoimmune diseases.

Choosing your production system



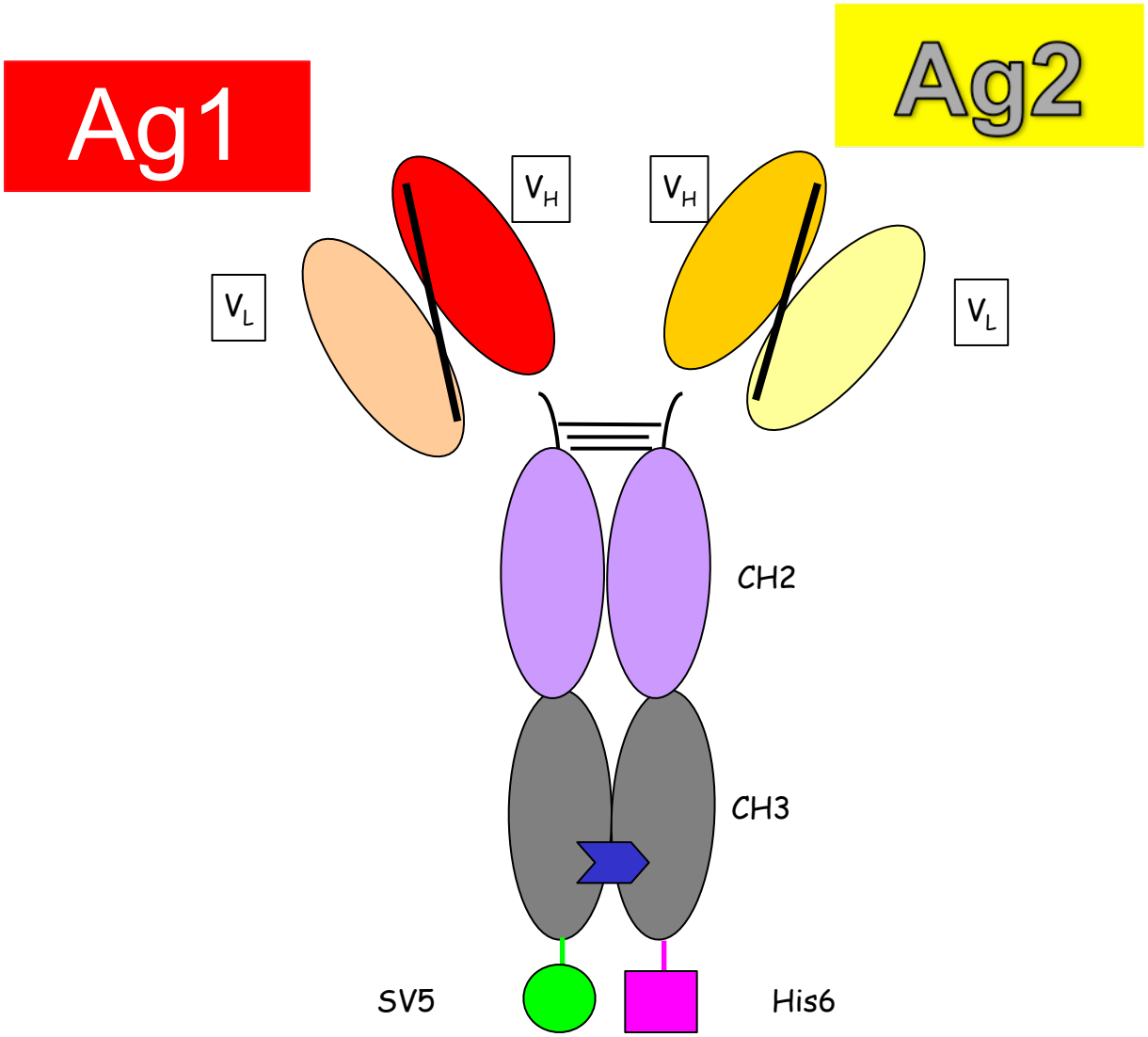
Antibody modifications

- Increase affinity
  - Chain shuffling
  - Point mutation

- Modification to Fc portion
  - Change Fc (IgG, IgM, IgA; mouse, rat, human)
  - Enhance CDC
  - Enhance ADCC

- Production of bispecific antibody

Bispecific antibodies



Antibody purification

- Using Fc portion
- Using specific Tag
- Using the antigen