Evolution of the immune systems. “To create is to recombine” (François Jacob 1977)

“Since the idea of the germinal layers (‘immune system’) was taken from the higher animals, and was then applied to the invertebrates, it was natural that misconceptions should arise, owing to such an anti-genealogical method.”
Elie Metchnikoff 1893

“Sociologists of science point out that the great advance of one generation may retard progress in the next. This is because each advance induces a mindset in the scientists that slants the interpretation of data and new speculations. Thus, we saw how the brilliant progress in antibody research early in the century led to later delays and false leads in the exploration of cellular immune reactions. (...) We must take care always to question our preconceptions, rather than taking the easier path of designing experiments to confirm them.”
(A.M. Silvertsein, Nature Immunology 2002, 3:105)
Selective pressures and constraints on the immune systems

- Should confer to each individual within a species the best chances of survival under changing environment conditions.

- Should be rapid and efficient at eliminating specifically the danger

- Should have diversity and flexibility, should be regulated (up and down regulation)

- Should be economical i.e. should not use too much genetic material, too many cells.

- Should not forget the previous encounter with pathogens,

- Should transfer of protection to the progeny could be useful.

- Should not react with self.
Selective pressures and constraints on the immune systems

- Should confer to each individual within a species the best chances of survival under changing environment conditions.
- Somatic adaptation

- Should be rapid and efficient at eliminating specifically the danger
  - innate components, constitutive elements

- Should have diversity and flexibility, should be regulated (up and down regulation)
  - Many solutions: gene families, many molecular categories, Treg

- Should be economical i.e. should not use too much genetic material, too many cells.
  - Somatic adaptations, combinatorial usage of elements

- Memory, transfer of protection to the progeny could be useful.
  - Clonal selection. Inheritable specificities. Maternal transfer of Antibodies

- Should not react with self.
  - Selection
  - peripheral tolerance

- (Modulations of the above are likely under the influence of life histories of the organisms)

Duplications, cooptation, combination, analogies, convergence, homologies, divergence
Early Metazoa

Cells

Soluble Discriminating receptors and effectors

Complement C', Prophenol ox. PPO

Diversification

Multiplication

NOD like R, Rig1--

Intracellular

Cellular Discriminating receptors

Signal

Conservation: kinases

phosphatases

NF-KB, Etc.

Extracellular

Diversification

Germ line duplication, polyplody

combinatorial assembly

Domains or peptides

Effectors

Soluble, surface

(sometimes = receptors)

Diversification

AMP

Effector life histories

Life histories

Epithelia

Organs

Fat body and alike

Gut

Constraints

Selection pressures

Innate immune systems of many Metazoa

Conservation: kinases

phosphatases

NF-KB, Etc.

1

Nature Reviews | Genetics
Early Metazoa

Soluble Discriminating receptors and effectors

Cellular Discriminating receptors

Intracellular

NOD like R, Rig1--

Complement C', Prophenol ox. PPO

Extracellular

Germ line duplication, polyploidy

NOD like R, Rig1--

Soluble, surface (sometimes = receptors)

Effectors

Conservation: kinases, phosphatases, NF-kB, Etc.

Signal

Conservation: kinases, phosphatases, NF-kB, Etc.

Germ line duplication, polyploidy

combinatorial assembly

Domains or peptides

Somatic diversification

RNA: alt splicing

combined

DNA: Mutations, gene conversion

Rearrangement: combinatorial usage of gene segments

AID, RAG

Individual random repertoires

All Vertebrates

Adaptive immune system

Most living Metazoa

LRR

Epithelia

Life histories

Constraint

Selection pressures

Cells

Innate immunity

Most living Metazoa

Adaptive immune system

2
Early Metazoa

Cells

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Germ line duplication, polyploidy

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RNA: alt splicing

DNA: Mutations, gene conversion

Rearrangement: combinatorial usage of gene segments

AID, RAG

Individual random repertoires

All Vertebrates

Adaptive immune system

...Lymphocytes

clonal selection

Specific memory

Autoimmunity? 

Selection

Central tolerance

Peripheral tolerance

T reg

Thymus

AIRE

GALT

Fat body Liver,

Phagocyte

Myeloid

Lymphoid

T, CTL, TH1, TH2, TH17, NK, B

Hematopoietic cells

Division of the work

Organs

Epithelia

Presentation

MHC I, II

Transcription factors

Organs

Most living Metazoa

Constellation of systems

Innate immunity

Constraints

Selection pressures

Life histories

Diversification

Conservation:

kinases, phosphatases, NF-KB, etc.
Early in evolution…

- **Simultaneous threats**: Viruses, bacteria, fungi, individuals of the same species, variants of self.

- **Simultaneous responses**: original diversity of immune mechanisms, receptors and effectors. Multiple evolutionary lines some shared between plants and animals. Ability to recognize self from non-self, danger signals.

- Restriction enzymes, RNA interference

- Soluble „receptors“ at the origin of proteolytic cascades (PPO, C‘)

- Phagocytosis, encapsulation, and subsequent destruction of pathogens (macrophages)

- **Inducible responses** often articulated around conserved domains and signalling cascades with up and down regulation (AMP, antibodies, CTL proliferation etc)
Elements

Constraints:
> a diverse but limited number of molecular categories
Protein domains membrane receptors of immunocytes in metazoa

- Lectins in Proto- and Deuterostomes
  - LRR
  - in Plants, Proto- and Deuterostomes
  - (Caterpillar, NOD, VLR, Toll, TLRs)
  - LRR

- PGRPs in Proto- and Deuterostomes

- Scavenger receptors in Proto- and Deuterostomes
  - SRCR

- EGF epidermal growth factor
  - CD36 (No crystal)

- Igfs Proto- (FREPs, DSCAM VCDBPs) and Deuterostomes
  - (Ig, TCR, NITRs, LITRs, KIRs, Fc-R, etc)

- CCP complement control proteins
  - in Proto- and Deuterostomes

- MHC Class I and II PBR
  - Gnathostomes

- ...Others

QuickTime™ and a TIFF (Uncompressed) decompressor are needed to see this picture.
Immunoglobulin domains

A B C D E F G rearrangement
A B C D E F G no rearrangement

V
Dimer interface; A'C, C', C'', F, G

I

C1
Dimer interface; A, B, D, E

C2

Restricted to gnathostomes?
JAM V domain residues binding reovirus

Diversification of receptors

Polymorphism

Modularity
Multigene families

Tandem duplications
Polyploidization
The CHIR (Chicken immunoglobulin-like receptors) gene family

Nikolaidis N. et.al. PNAS 2005;102:4057-4062
Combinatorial associations
Combination of Light and Heavy chains depending on the number of germ line genes (variable among species) the final number of combinations can be $10^6$ to $10^7$

CL and CH numbers can also vary and bring extradiversity

---

**Mouse heavy chain germline DNA**

- VH gene cluster
- DH
- JH
- Cμ
- Cδ
- Cγ3
- Cγ1
- Cγ2a
- Cγ2b
- Ce
- Cα

**Mouse kappa light chain germline DNA**

- Vk gene cluster
- Jk
- Ck

**Mouse lambda light chain germline DNA**

- Vλ2
- Jλ2
- Cλ2
- Jλ4
- Cλ4
- Vλ1
- Jλ3
- Cλ3
- Jλ1
- Cλ1
The cytosolic sensors Nod1 and Nod2 and Toll-like receptors (TLRs) activate defense signaling pathways in response to microbial stimuli.

Structure and cellular location of TLRs and NOD1 and NOD2.

Strober W et al. (2006) Signalling pathways and molecular interactions of NOD1 and NOD2

*Nat Rev Immu* 6: 9–20 doi:10.1038/nri1747
Combinatorial associations
+ Somatic adaptations
A. *Dscam* contains constant and variable exons.
Two enzyme of the same family APOBEC (Apolipoprotein B mRNA Editing enzyme, Catalytic polypeptide) involved with the preceding somatic events:

copy paste or gene conversion

cytosine deaminase 1 (CDA1) or 2 (CDA2),

Pancer Z et al. Nature Imm. 2007 8:647-656
Schatz D Nature Imm. 2007 8:551-553
Two variable lymphocyte receptor genes of the inshore hagfish VLRa and VLRB are located far apart on the same chromosome. Kasamatsu J et al Immunogenetics. 2007 Apr;59(4):329-31. Epub 2007 Feb 14

“VLRA and VLRB anticipatory receptors are expressed by separate lymphocyte populations by monoallelic VLRA or VLRB assembly, together with expression of cytosine deaminase 1 (CDA1) or 2 (CDA2), respectively. Distinctive gene expression profiles for VLRA(+) and VLRB(+) lymphocytes resemble those of mammalian T and B cells” Guo P et al Nature. 2009 Jun 11;459(7248):796-801.
Immunoglobulin domains

A B C D E F G rearrangement

A B C D E F G no rearrangement

V

CDR1

CDR3

CDR2

Dimer interface; A'C, C', C'', F, G

I

C

C1

C2

Dimer interface; A, B, D, E

Restricted to gnathostomes?
• RAG 1 and 2 (Chr 11p) (Recombination activating gene)
• Rearrangement V(D)J
• Present in Echinoderms, not in hemocytes, expressed early during gastrulation. Ancestral form: a transposon, Transib, identified in *Drosophila*?
• Rast J et al. PNAS 2006 103: 3728-3733
Agnathan VLR gene

Rearrangement with flanking LRR cassettes

Lymphocyte recombinatorial genes

Cell-bound anticipatory receptors

Humoral effector molecules

Gnathostome antibody genes

Light chain

RAG-mediated rearrangement

Heavy chain

Could AID be involved in the IgSF somatic modifications in Gnathostomes?

**AID (Hs chr 12p): Activation-Induced (Cytidine) deaminase**
**One paralog of the APOBEC family**
**(Apolipoprotein B mRNA Editing enzyme, Catalytic polypeptide)**

- Removes the amino group from the cytidine > uridine
- Involved in three separate somatic diversification processes: Somatic hypermutation, gene conversion, Ig heavy chain class switch within IgSF members of Gnathostomes.

- A AID homolog is involved in an analogous process

- In Gnathostomes two enzymes involved with the preceding somatic events:
  AID and RAG
Diversification of immune receptors

Many different processes selected during evolution can make the number of receptors much larger than the number of genes that encode them

Population level
• Polymorphism (receptors and effectors: lectins, C' related, AMPs, srcr, Ig, TCR, MHC etc)

Individual level
• **Peptides** Combinatorial association of polypeptide chains: Ig H.L, TCR \(\alpha\beta\gamma\delta\), TLRs, PGRPs, DSCAM,…
• **Nucleic acids**
  • **RNA**
    • Alternate splicing: e.g. PGRPs FREPs, DSCAM, Fester…
  • (Arthropods, Mollusks, Echinoderms, Prochordates, Vertebrates)
• **DNA**
  • Somatic rearrangement: Ig, TCR (combinatorial joining) (Gnathostomes)
  • Somatic gene conversion: Ig Vertebrates. LRR Agnathans?
  • Somatic mutations: Ig from sharks on. Mollusks?
  • Switch Ig heavy chain

Individualization of responses
Problems of expression
Autoimmunity?
Selection
MHC Class I and Class II pathways of presentation

Class I: LMP TAP self, viruses
Class II: external, lysosomes

Unipotential lymphocytes, clonal selection
Lymphocytes
Cytokines (Upd-3)

Lymph glands

Circulating PRRs
GNBPs/PGRPs

Serine proteases/
serpins

Toll

PGRP-LC

Domeless

Toll pathway

Imd pathway

JAK/STAT pathway

Fat body

Antimicrobial peptides,
iron sequestration, DIMs,
serine proteases/serpins,
stress factors (turandots),
opsonization (TEPs),
clotting factors (fondue)

Encapsulation
Phagocytosis
Coagulation
Melanization

HEMOLYMPH

Mammals

Insects

MHC class I and II molecules

From *Immunity: The Immune Response in Infectious and Inflammatory Disease* by DeFranco, Locksley and Robertson

“In the urochordate-cephalochordate ancestor, I propose that the ancestor of MHC molecules presented hydrophobic peptides (including leader peptides of self and non-self origin) to an ancestral CD94 receptor (a lectin), with a role in stress and/or danger detection. Consistent with this, Flajnik et al. have previously proposed that ancestral MHC molecule(s) derived from heat shock proteins (Hsp), which have particularly high affinities for hydrophobic peptides. Although the proposal by Flajnik et al. was based on tenuous sequence homologies, and later elucidation of an Hsp crystal structure revealed that Hsps and MHC molecules bind to peptides in very different fashions, this type of hypothesis remains interesting, if only because Hsps do behave as danger signals when they are released in the extracellular milieu, in line with a recent hypothesis regarding the hydrophobic nature of danger signals”.

Thymus and intra thymic selection
Planche 3.10
Origine du thymus chez les Vertébrés.
Schéma de la région branchiale.
AIRE

SAND: Sp100, AIRE-1, NucP41/75, DEAF-1DNA binding fold
Bromo: 4 helix structure, chromatin regulation.
PHD: Plant homeo domain-Zinc finger
HSR: Homogeneously Staining Region= N-terminal beta helix loop helix (HLH)

AIRE homolog genes have been isolated in all gnathostomes but so far not in cartilaginous fish
Gregersen and Behrens *Nature Reviews Genetics* 7, 917–928 (December 2006) | doi:10.1038/nrg1944
Clonal selection

Coico R and S Sunshine 2009

web.rcai.riken.jp/en/labo/antigen/
Origin of costimulators, regulators…
Antigen Presenting Cell

- CD200R
- CD80/86
- CD58
- CD48
- SLAM6
- SLAM7
- SLAM
- CD84
- CD40
- CD166
- CD47
- MHC ClassII

CD200

CD28
- CTLA4
- BTLA

CD2

SLAM6

SLAM7

SLAM

CD84

CD40L

CD6, 5

SIRPγ

CD4

TIM4

TIM1

ICAM

CD45

CD11-18

CD22

NK or CTL (killer)

Target cell

Immunological synapse

T Cell

CD155

CD155

NK or CTL (killer)

CRTAM

CD96

Ig V-fold domain

Ig C1 -L domain

Ig C2 domain
(Du Pasquier L. CTMI (2000) 248:159-185

**C. i CTX/JAM-L**

**C. i Nectin-L**

**C. i CD 166-L (CD6 ligand)**

**B. s. FuHC (IgSF4-L)** (De Tomaso et al. 2005 Nature 438 :454-9)

**B. l. VCnp. (CTX/JAM-L)** (Yu et al 2005 JI 174: 3493-3500)

**B. l. VCTX-L**

**CD 47-L (SIRP ligand)** (Sato A et al 2003 Immunogenetics 55:423-7)
### Position on human chromosomes of homologs of Invertebrates IgSF members

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<tr>
<th>Chr 1p13/q22</th>
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<th>Chr11q23-25</th>
<th>CH21q21-22</th>
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<td>⭐️ Igsf4 nectin</td>
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<td>LRC</td>
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Chromosomal regions in Tunicates and Vertebrates (Homo): * = IgSF

Homo 19q13

Ciona 10

Botryllus

Fester (VCRL1-L)

Ci Nec 2 BCKDHA IgSF

Ciona 4

VCRL1 IgSF CD22i VCRL1 VCRL1 CD66L CBLc Ros0264 VCRL1 IgSF BCL3 IgSF Ci Nec1 Kirrel1

VCRL1 IgSF CD22i VCRL1 VCRL1 CD66L CBLc Ros0264 VCRL1 IgSF BCL3 IgSF Ci Nec1 Kirrel1
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Cells

Selection pressures

Epithelia

Constraints

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Complement C', Prophenol ox, PPO

Germ line duplication, polyploidy

Effector
Soluble, surface (sometimes = receptors)

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Intracellular

NOD like R, Rig1--

Coreceptors Costimulators ITIM ITAM

Extracellular

Signal

Conservation: kinases phosphatases
NF-KB, Etc.

Conservation:
kinases phosphatases
NF-KB, Etc.

Cellular Discriminating receptors

Germ line duplication, polyploidy

Combinatorial assembly
Domains or peptides
Polymorphism

Somatic diversification
RNA: alt splicing combinatorial
DNA: Mutations, gene conversion
Rearrangement: combinatorial usage of gene segments

AID, RAG

Indi...
The Gnathostomate (Jawed Vertebrates) adaptive immune system in evolution
Shark V gamma undergoes somatic hypermutation

HYPOTHETICAL JAWED VERTEBRATE 'UR' MHC

- Class I, II, III genes linked

TELEOST

- Loss of class II genes (including UDR linkage to class I) by translocation or differential inactivation after polyploidization

- Loss of class III immune genes

XENOPUS

- Highly conserved MHC conserved with humans
- Class I, class II, TAP, pentosamine genes tightly linked (Adaptive MHC)
- MHC I in class I region
- IgSF/V3 MR-like receptors in class III region

CHICKEN

- Compaction of genome including MHC; loss of "non-essential" genes especially LMP from the genome
- Expansion of non-classical class I/II but from MHC proper
- CD1 genes linked to BMM
- C-type lectin MR receptor genes linked to MHC

HUMAN

- Translocation of class I and subsequent expansion
- Translocation of MHC1 to chromosome 16

Immunoglobulin light chains of Vertebrates
Immunoglobulin isotypes in Vertebrates

Ohta y and Flajnik MF PNAS 2006 103:10723-8
Zhao Y et al PNAS 2006 103:12087-12092
One more somatic event, a late comer: Ig heavy chain class switch
Germ line rearranged V  
Early ontogeny  
Mutated later  

Microbicidal peptides

Multiple C3

"Innate" γδ T and B cells