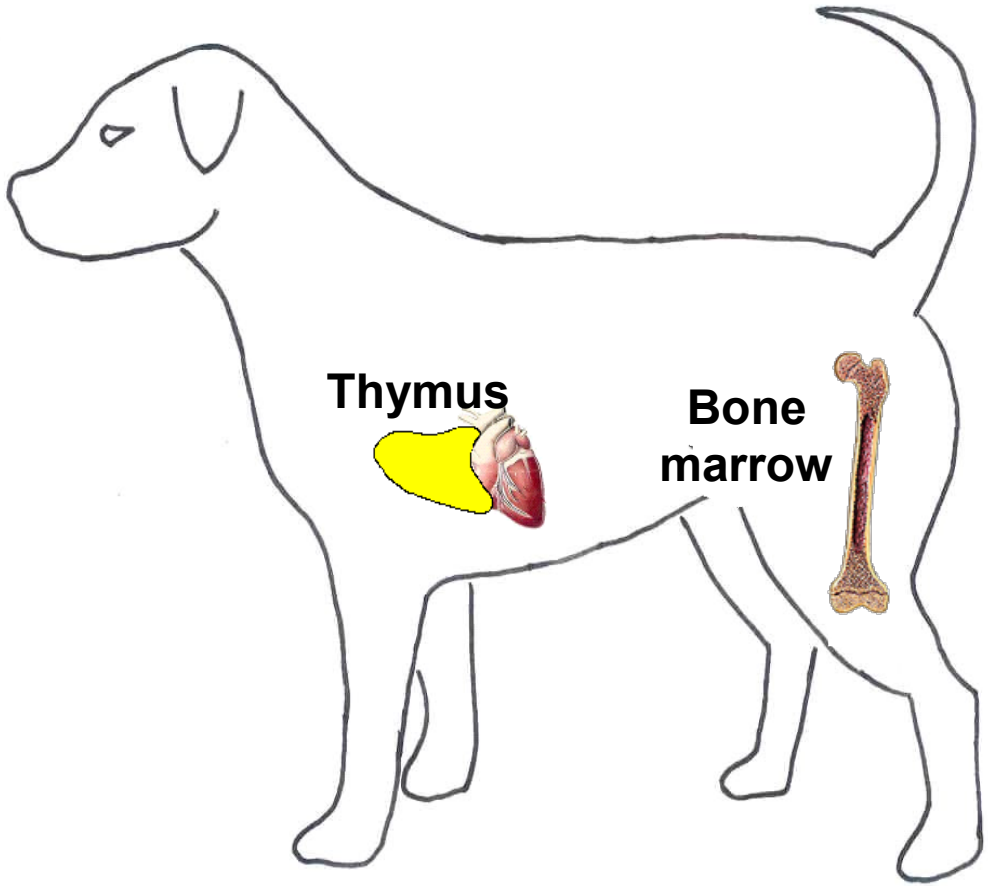
Royal Veterinary College
University of London

MSD ANIMAL HEALTH EXPERTISE 2023

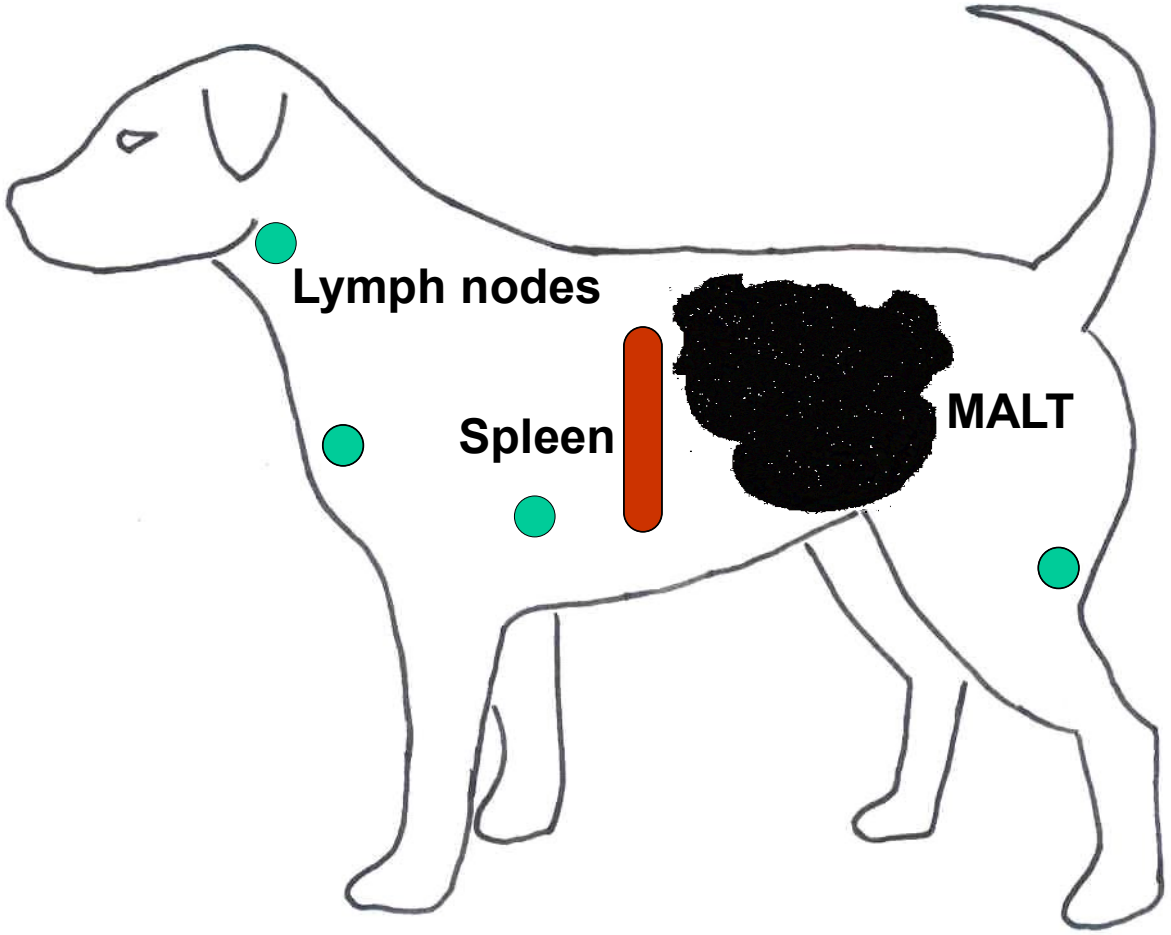
Immunosenescence in companion animals: Development and deterioration of the immune system

Brian Catchpole,
BVetMed MSc PhD FRCVS

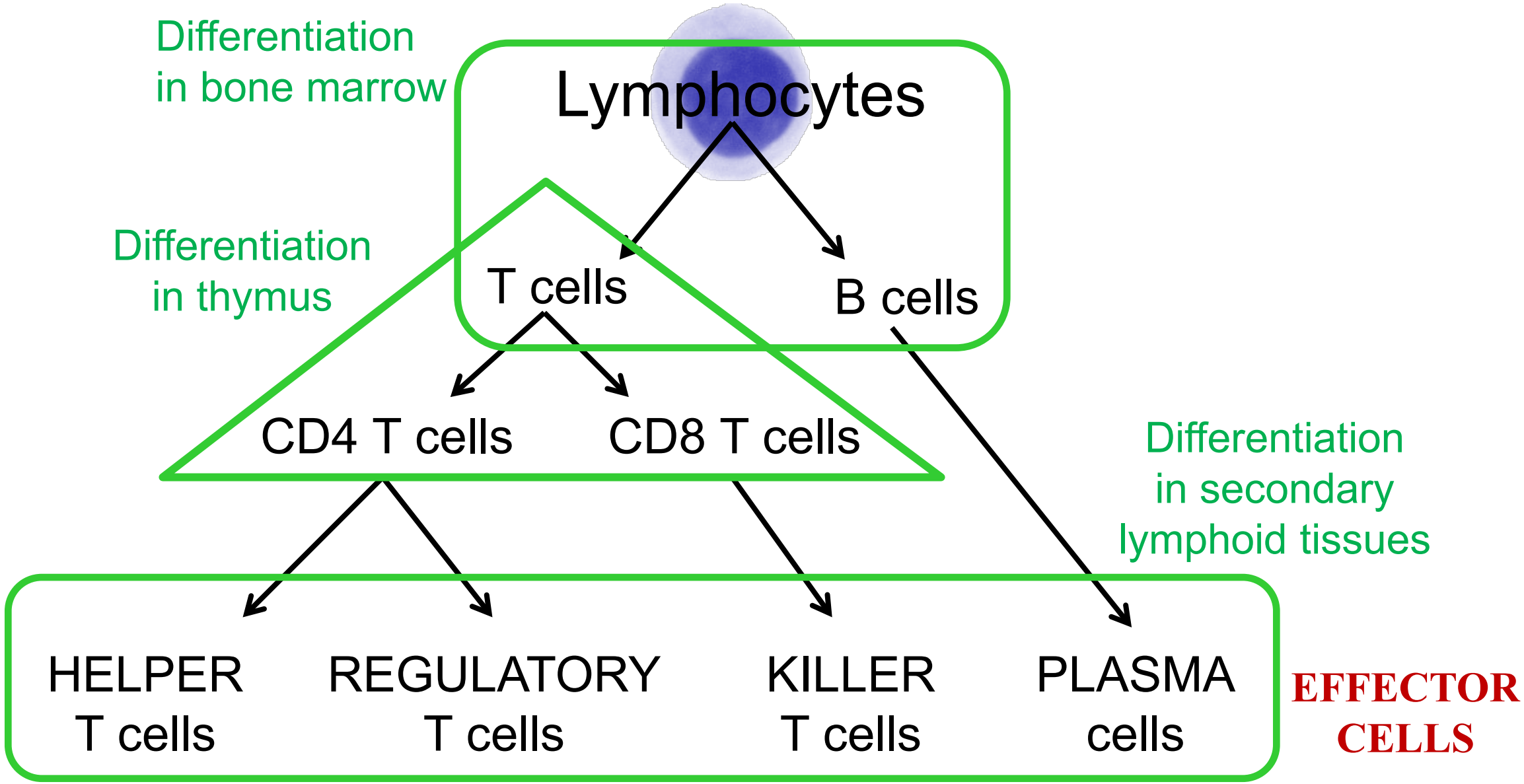
Primary lymphoid organs: Lymphocyte development



Secondary lymphoid organs: Lymphocyte response to infection / vaccination



The lymphocyte family tree

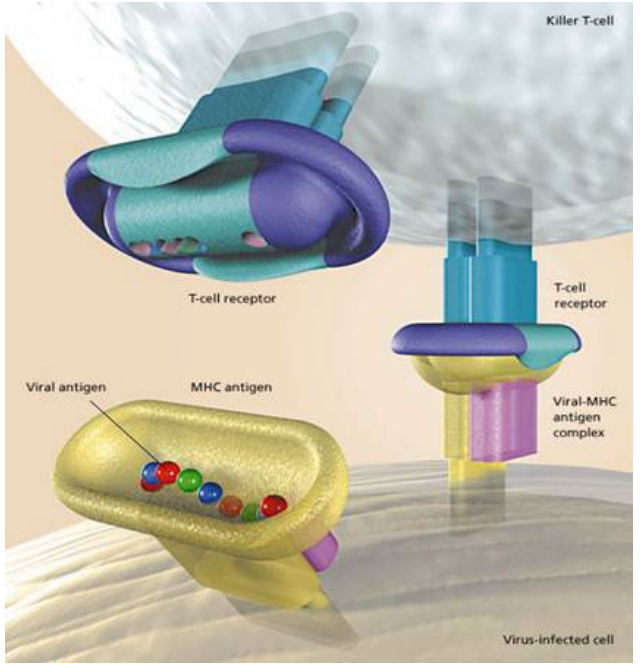


The secret of a successful adaptive immune system:

- Each individual lymphocyte expresses a unique antigen receptor which is highly specific.
- There are millions of lymphocytes and therefore millions of different shaped receptors
- This huge diversity of antigen receptors ensures that no foreign organisms will go undetected

Recognition of antigen by T cell receptor

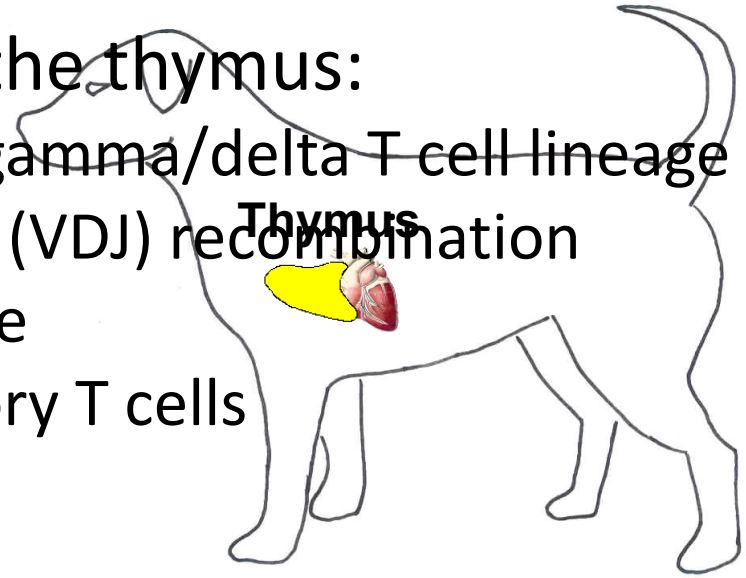
- T cells recognise digested fragments of antigen (peptides) displayed on the surface of other cells
- These peptide epitopes are presented by carrier molecules known as Major Histocompatibility Complex (MHC)
- The ‘immunological synapse’ between innate and adaptive immune responses



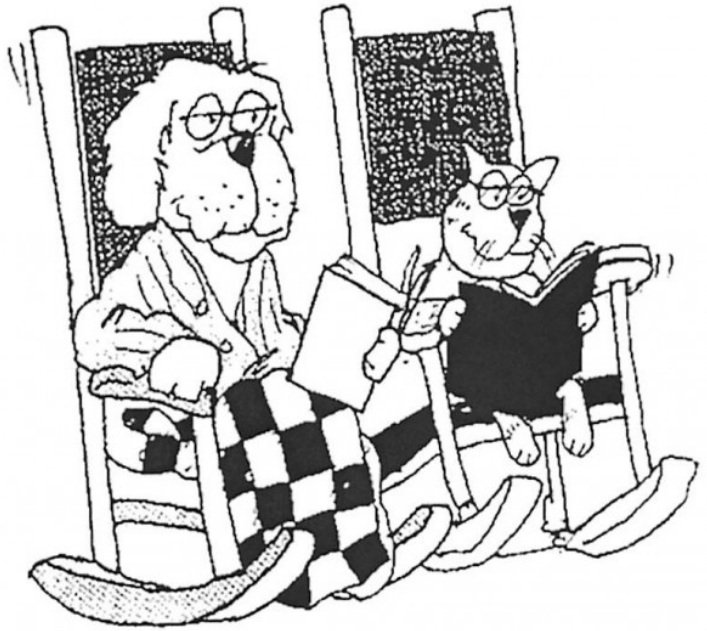
T cell development

- Lymphoid precursors in the bone marrow that become committed to the T cell lineage must travel to the thymus to complete their education
- They must pass several ‘tests’ before being allowed to enter the general circulation for immune surveillance

- Several important processes occur in the thymus:
  - Commitment to alpha/beta rather than gamma/delta T cell lineage
  - Development of a unique TCR by genetic (VDJ) recombination
  - Commitment to CD4+ or CD8+ phenotype
  - Self tolerance and generation of regulatory T cells

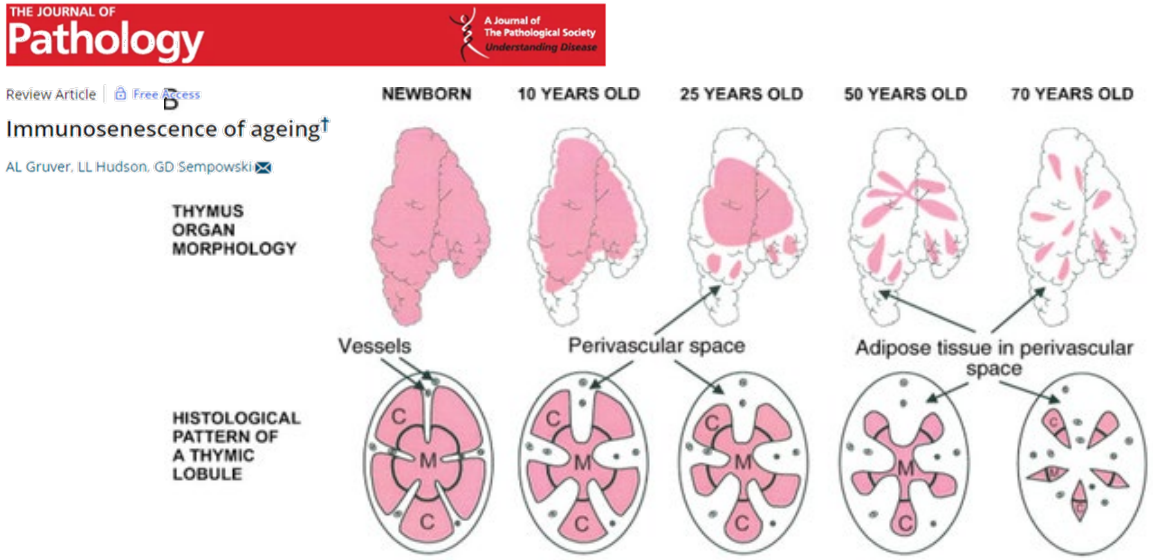


Thymic output and impact on immune function / immunosenescence



Thymic involution and atrophy

- The thymus undergoes an age-associated involution.
- Leads to a reduction in the production of naive T cells for recruitment into the circulation.
- One of the main contributory factors to the loss of immune function in old age.

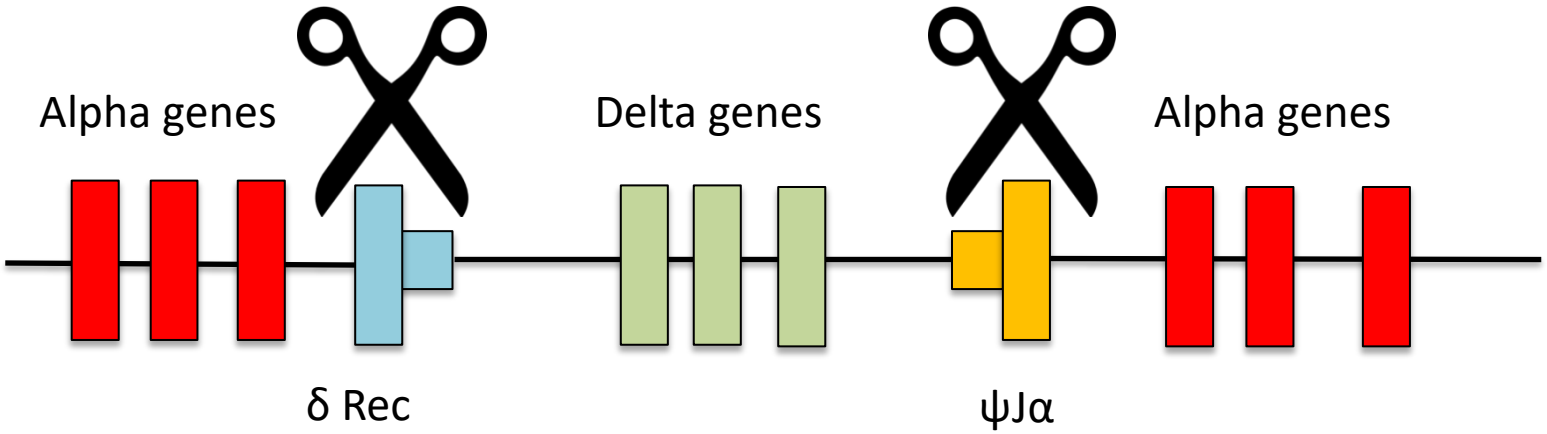


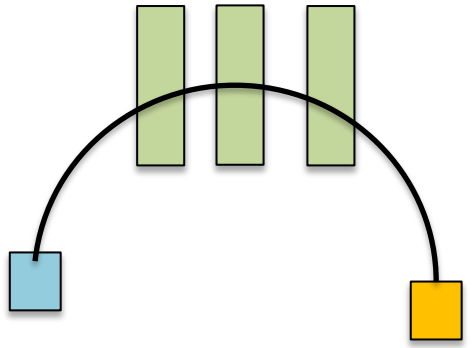
Immunosenescence

- The gradual deterioration in immune function associated with ageing.
- Geriatric individuals have a reduced capacity to mount an effective immune response.
- In humans - linked to reduced responses to vaccination and increased susceptibility to infectious and neoplastic diseases.

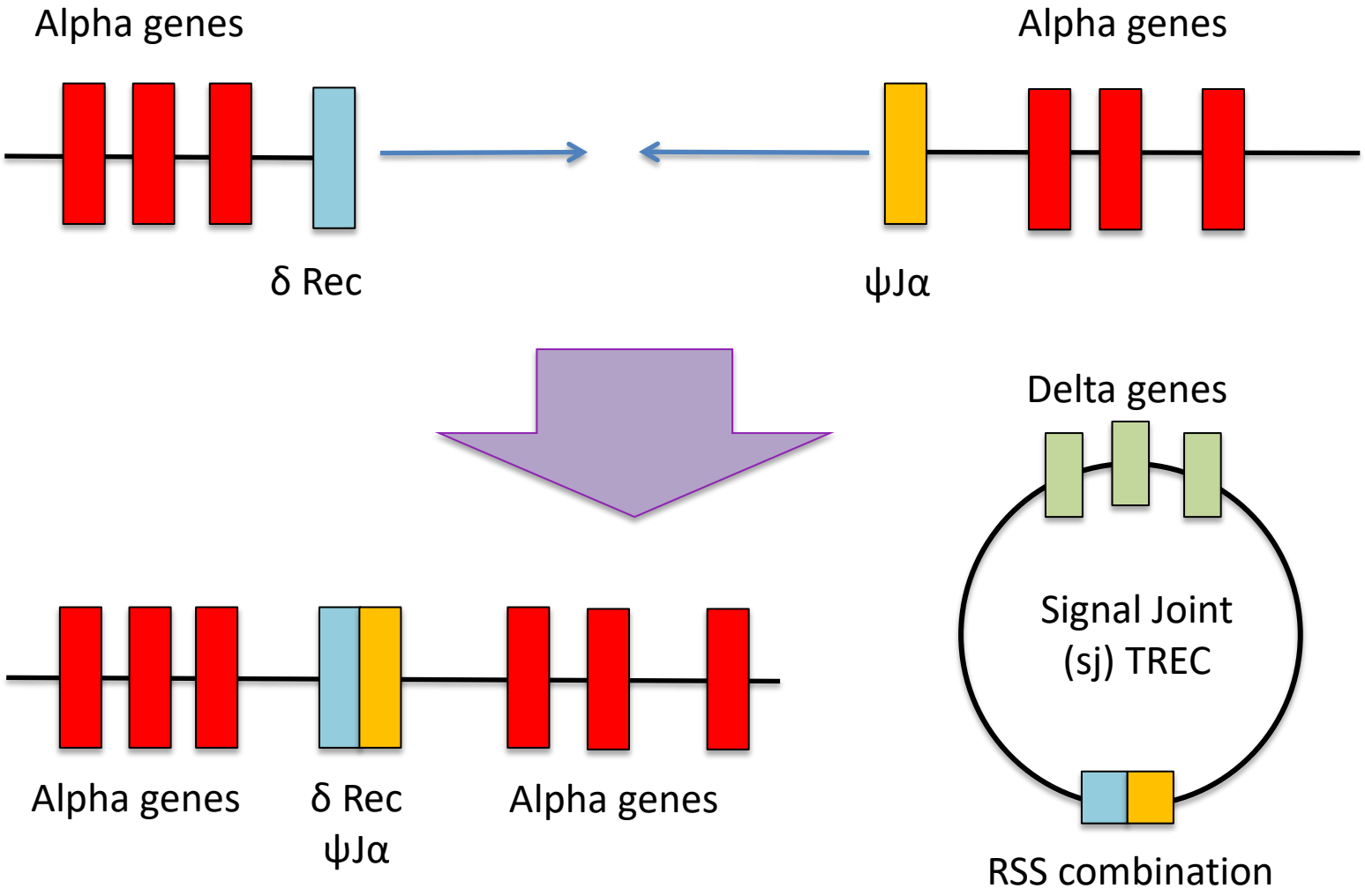
How can we measure thymic output ?

T-cell receptor alpha gene rearrangement

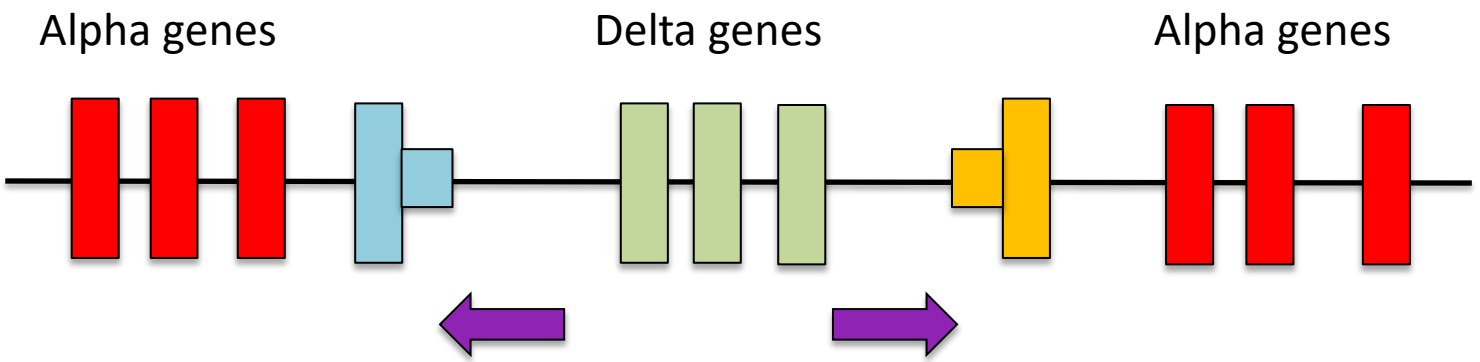




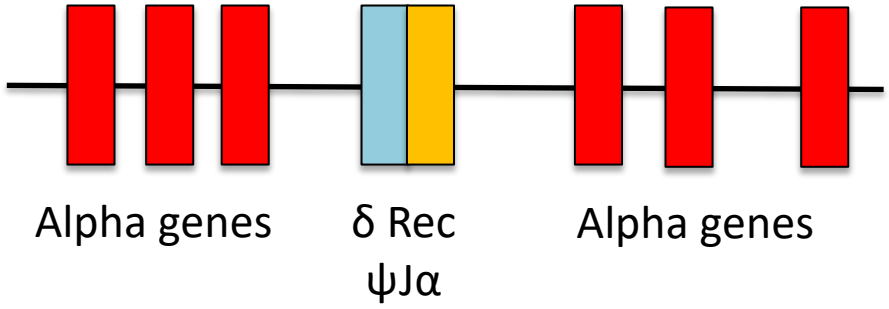
Sj-TREC: a by-product of T-cell development and ‘marker’ of recent thymic emigrants (RTEs)

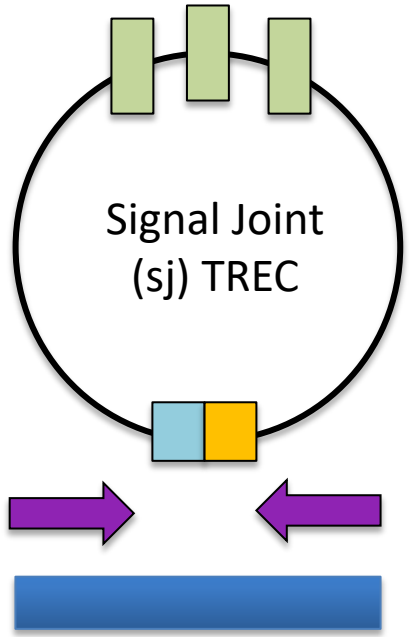


Sj-TREC PCR: a biomarker of thymic output



No PCR product



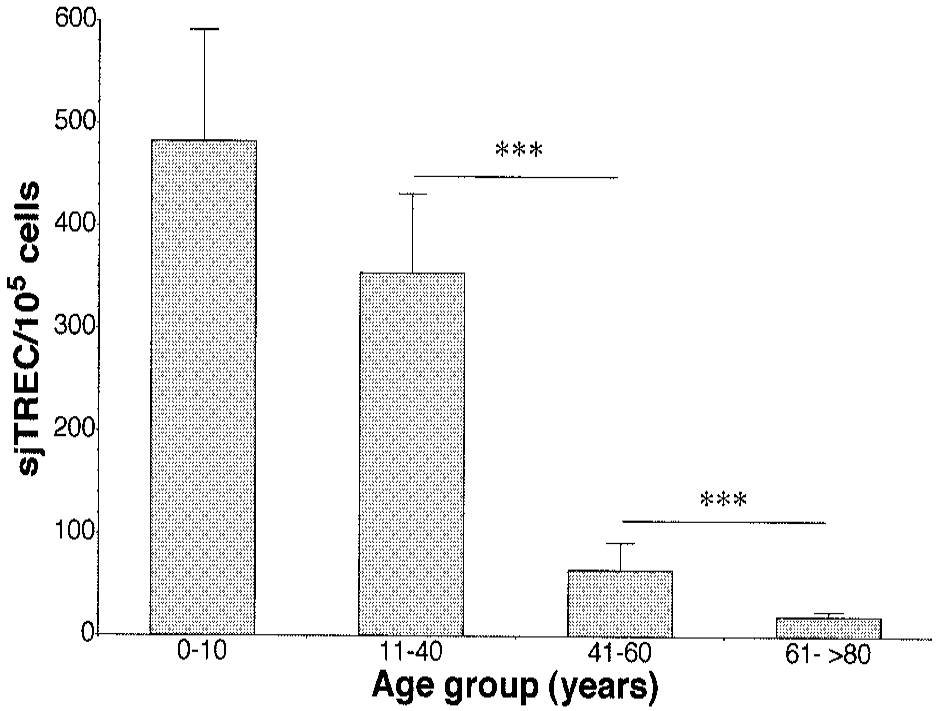


PCR product

Immunosenescence in humans

Measurement of sj-TREC in humans has shown that thymic output declines with age.

However, as sj-TRECs are still present at low levels later in life this suggests thymic output continues into old age.



Geenen et al. J Endocrinol. 2003: 176: 305-311.

Canine longevity

Small breeds live longer than large breeds.

13 to 14 years median life expectancy

6 to 7 years median life expectancy

Are there breed differences in immunosenescence?

Project aims

PLOS ONE

RESEARCH ARTICLE

An Age-Associated Decline in Thymic Output Differs in Dog Breeds According to Their Longevity

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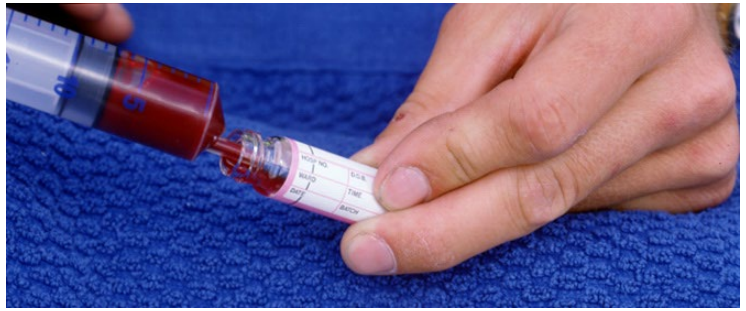
- To measure sjTREC values in dogs of different ages
  - To evaluate diversity of sjTREC values in young, middle-aged and older Labradors
  - To compare long-lived (e.g JRT) and short-lived (e.g Great dane, BMD) breeds

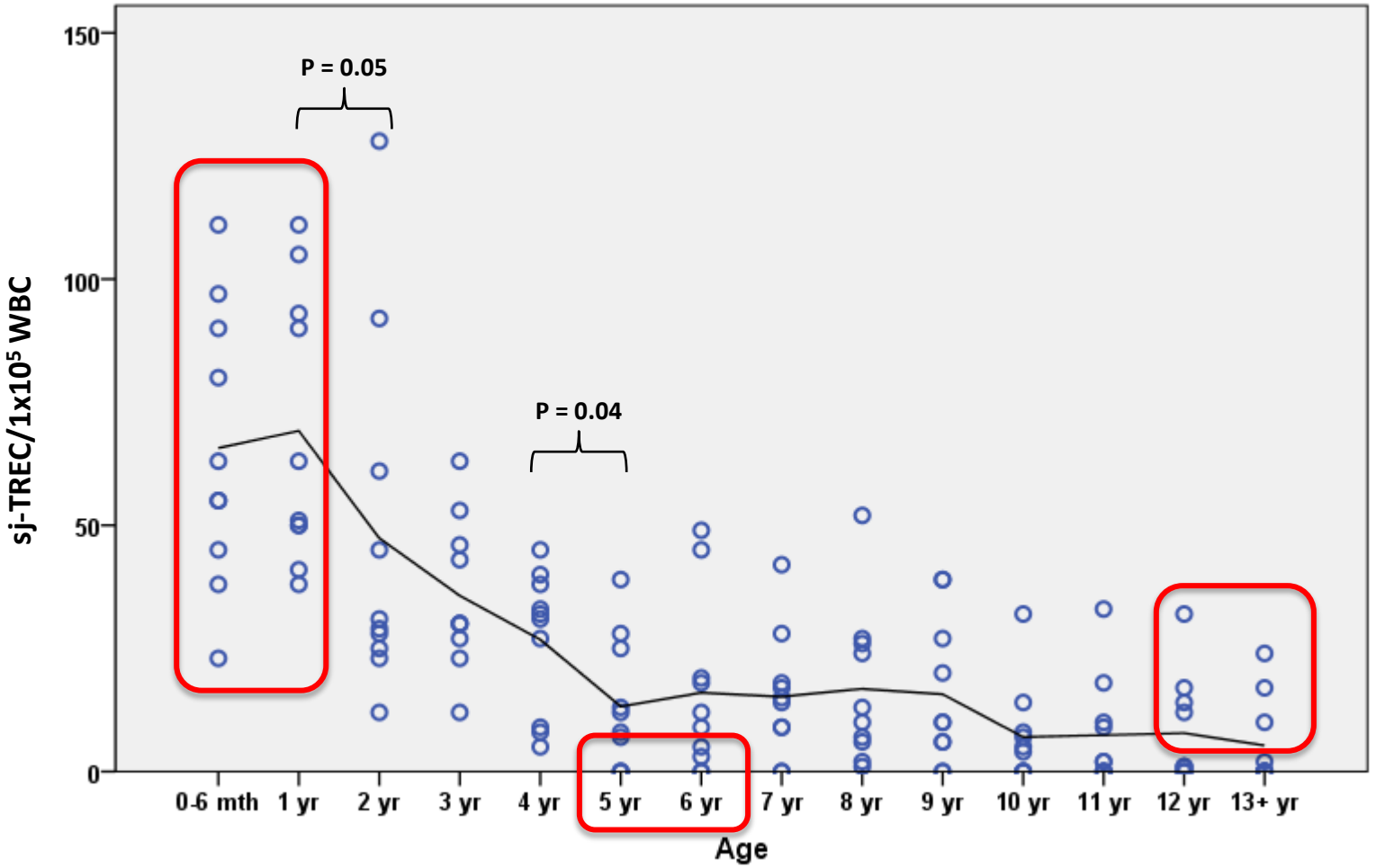
Methods

Extract genomic DNA from EDTA blood samples



Measure sj-TREC by real-time PCR

Sj-TREC in Labrador retrievers of different ages

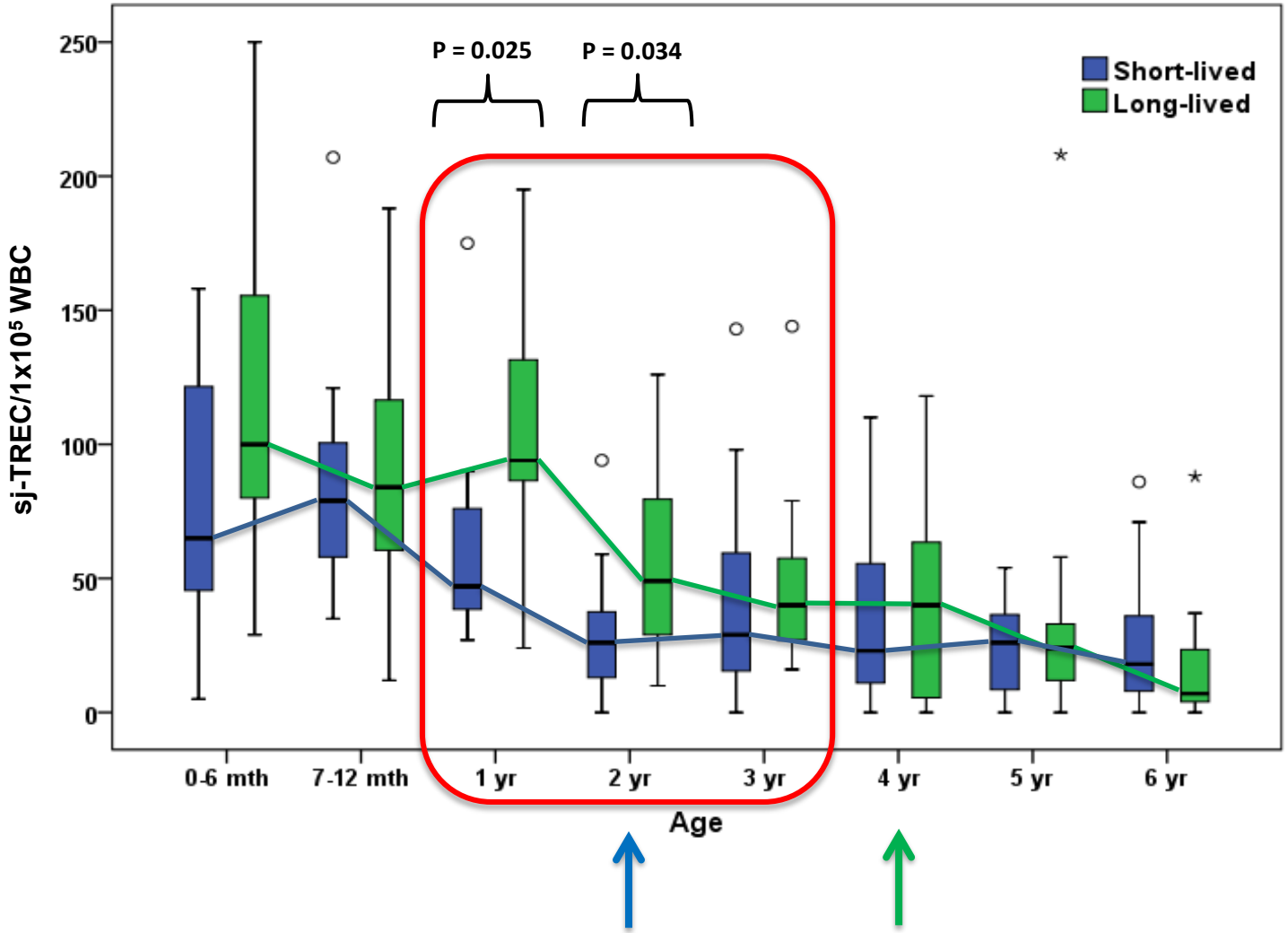


Considerable individual variation in young dogs

Some dogs seems to maintain thymic output into old age

Some dogs seems to show a premature decline

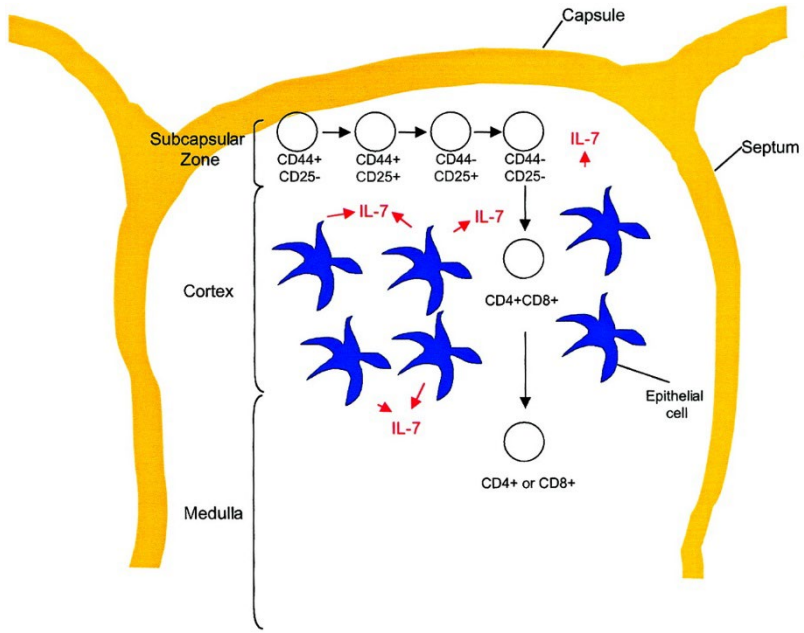
Differences in sj-TREC between short and long-lived breeds



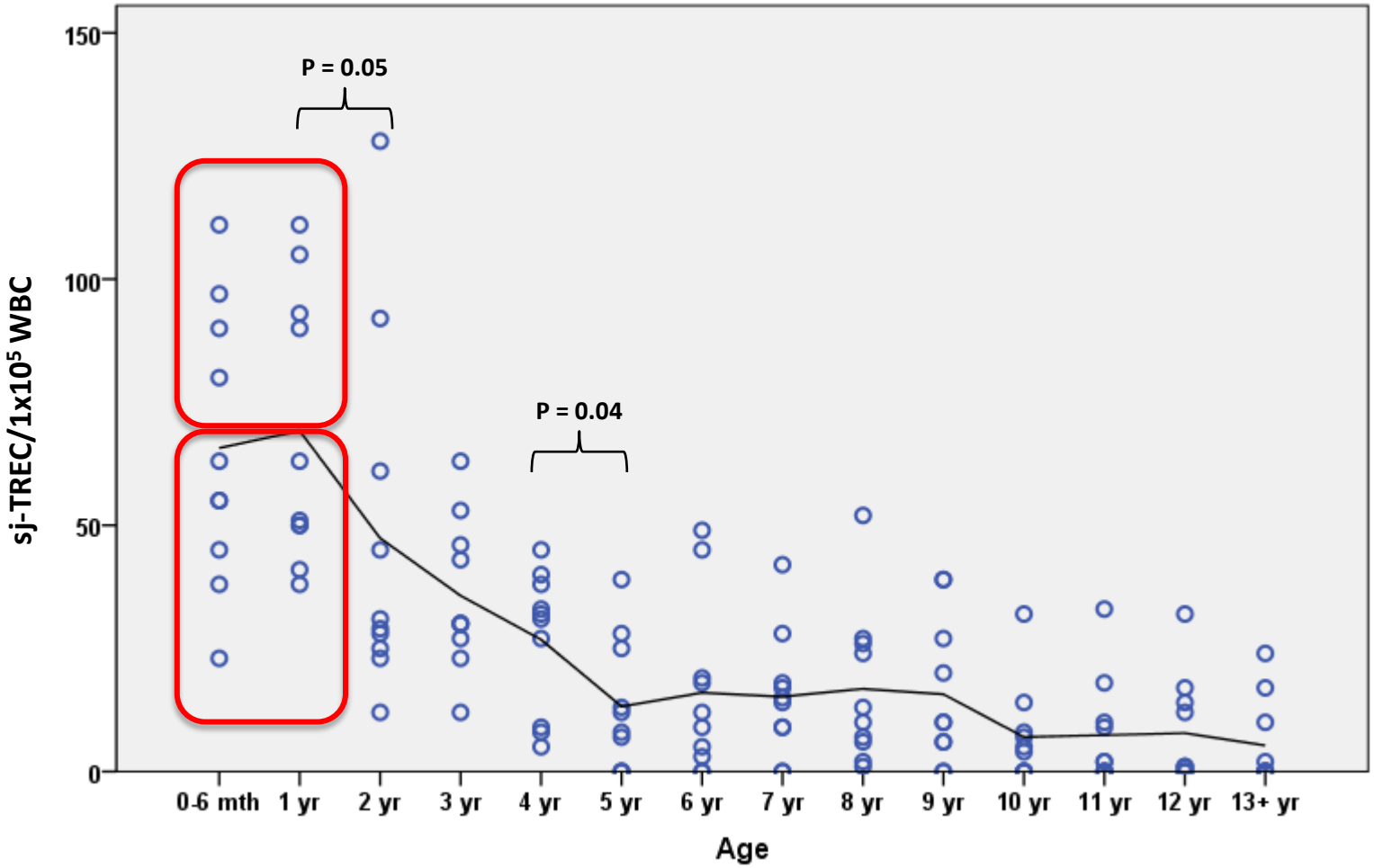
Short-lived breeds seem to go into decline at an earlier age

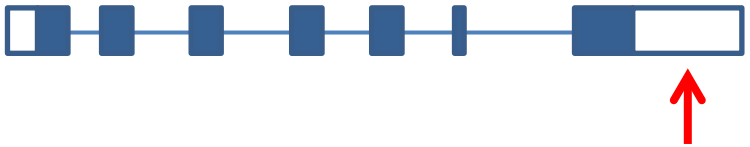
Variability in sjTREC

- Why is there such variability in Labrador dogs <1 yr old ?
- Why do some dogs seem to lose their thymic output in middle age ?
- What determines whether geriatric dogs maintain thymic output ?
- GENETICS?
- Interleukin 7 is an important cytokine for T cell development
- IL7 / IL7R are obvious candidate genes



Sj-TREC variability in young Labrador retrievers





IL7R variants

Contents lists available at ScienceDirect
Developmental and Comparative Immunology
journal homepage: www.elsevier.com/locate/dci

Polymorphisms in the canine *IL7R* 3′UTR are associated with thymic output in Labrador retriever dogs and influence post-transcriptional regulation by microRNA 185

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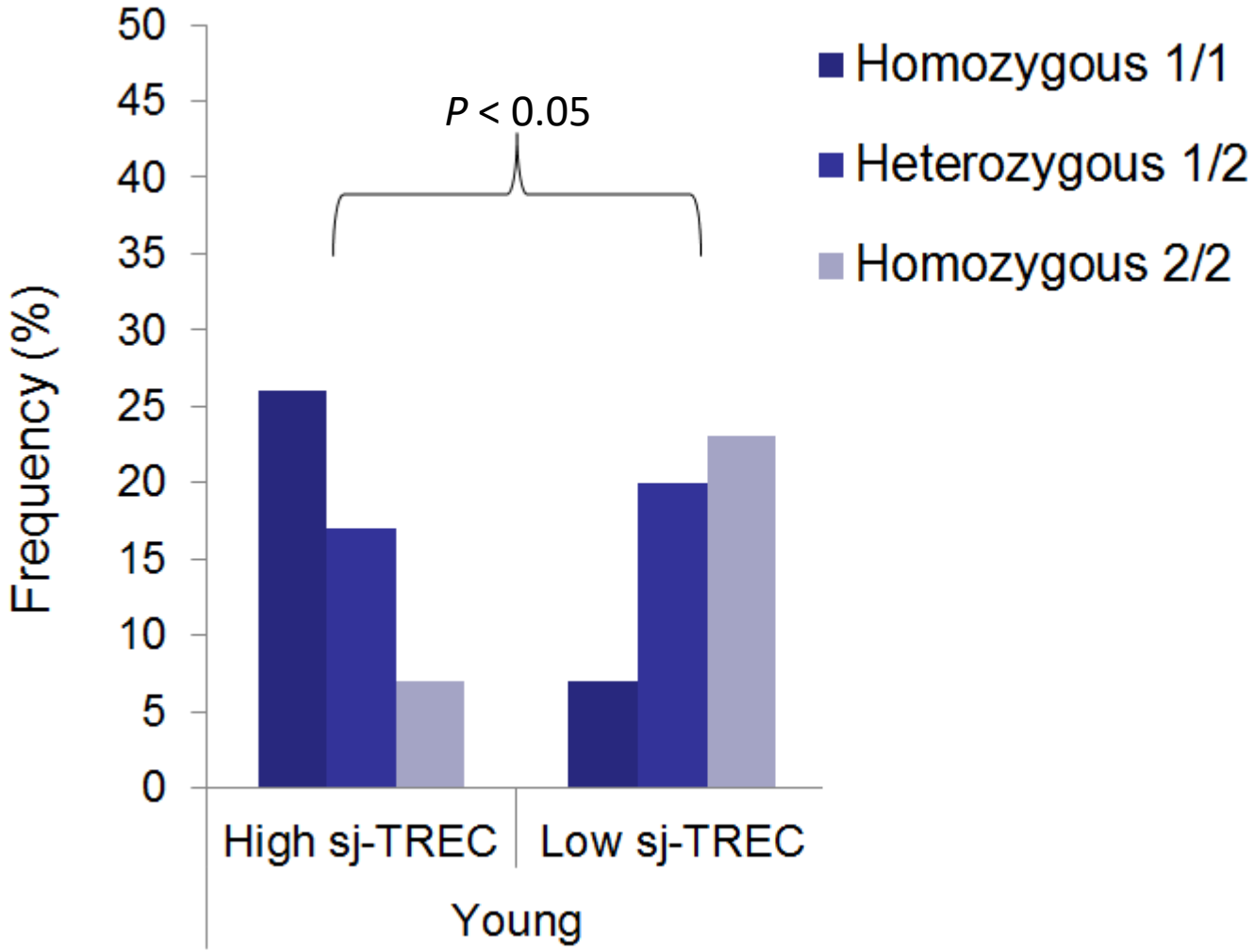
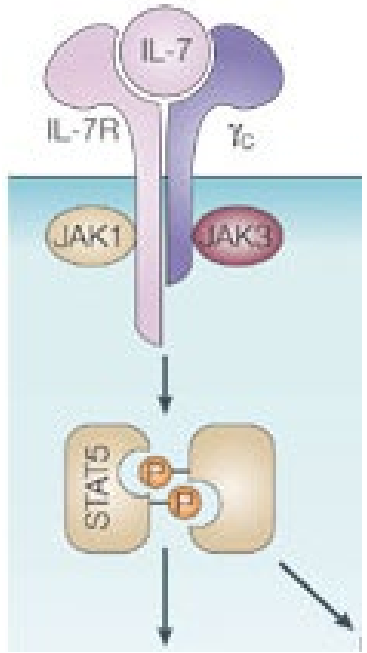
[a] Department of Pathobiology and Population Sciences, Royal Veterinary College, North Mymms, Hertfordshire, UK
[b] Department of Comparative Biomedical Sciences, Royal Veterinary College, London, UK
[c] Health and Wellbeing Academy, Postgraduate Medical Institute, Anglia Ruskin University, Chelmsford, Essex, UK



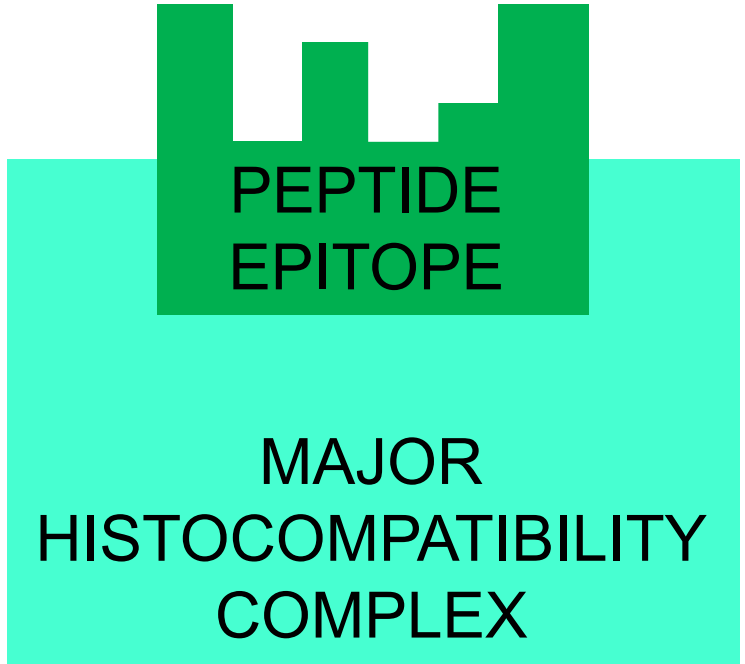


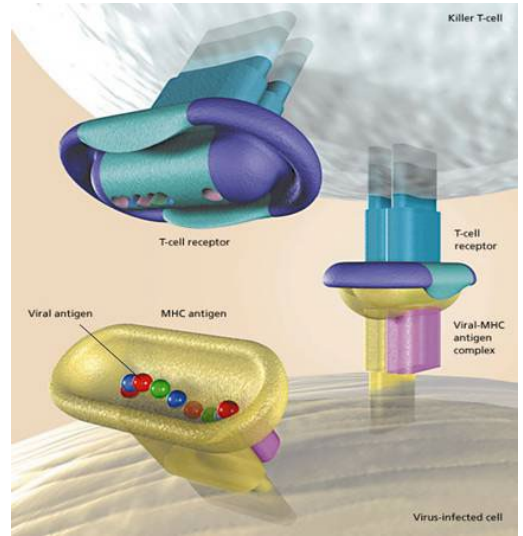
Thymocyte proliferation & differentiation

T cell repertoire

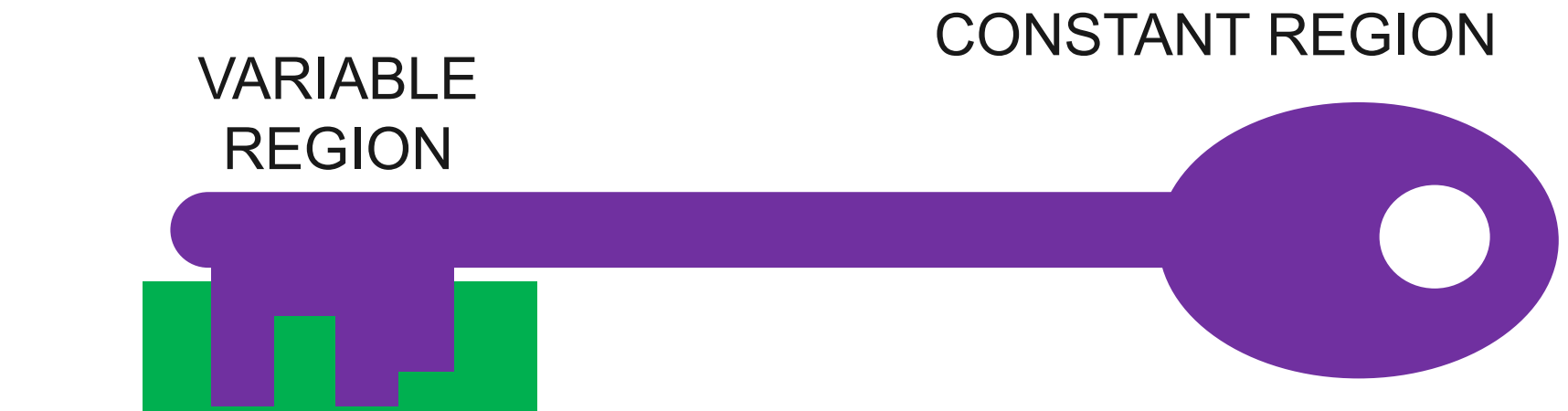
- There is great diversity in the T cell repertoire for detecting infection (foreign antigenic peptides)
- Generated by random selection of Variable and Joining segments of TCR genes in thymus
- Peripheral T cell repertoire changes over time with selection of memory T cells and potentially loss of naïve T cells exacerbated by failure to repopulate from thymus
- Ability to respond to re-infection (or booster) probably maintained but poor responses to novel antigenic challenges

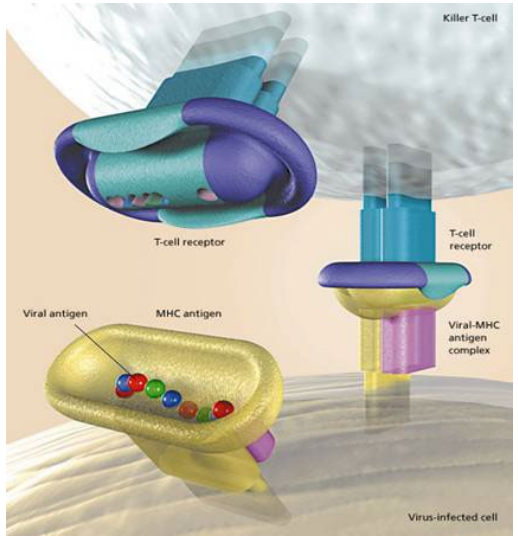
Antigen receptor development
The lock & key model



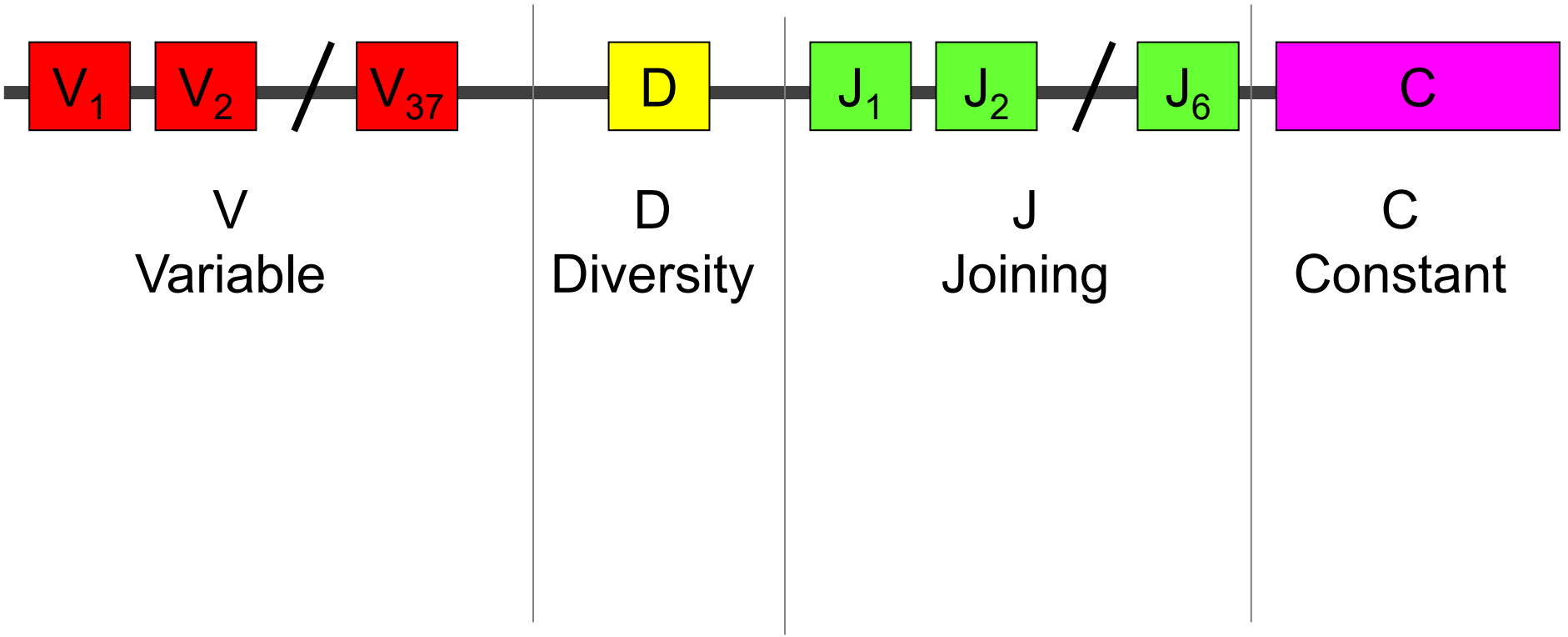


Antigen receptor development
The lock & key model



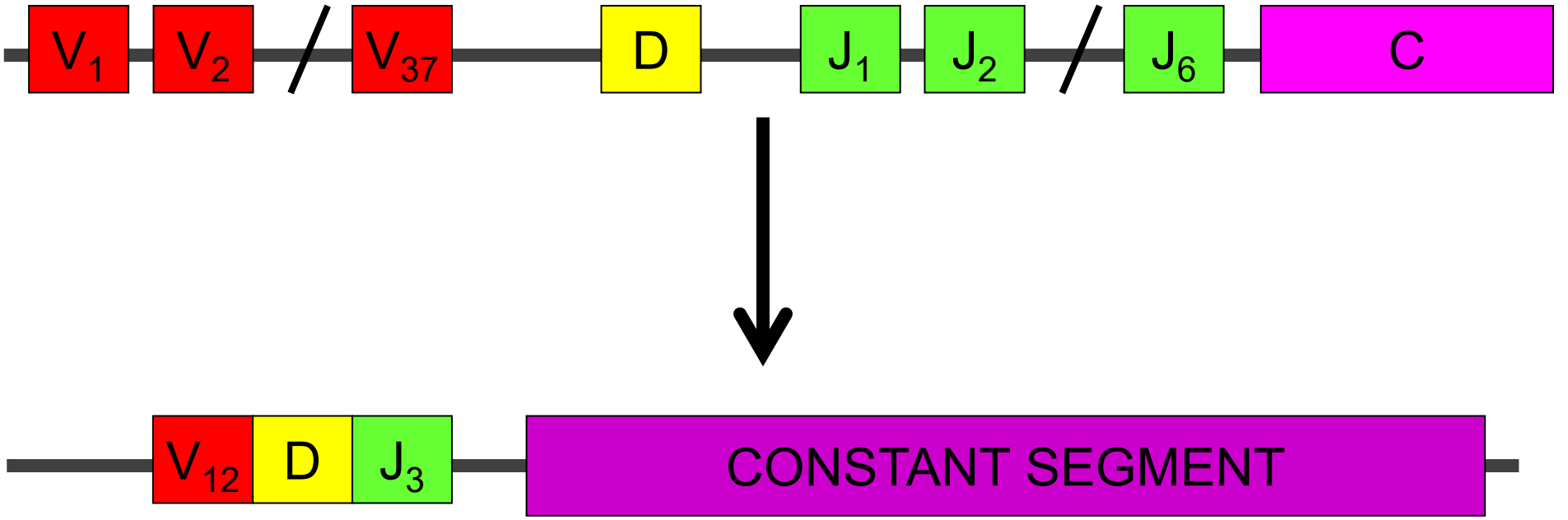


Variable region of TCR determines antigen specificity



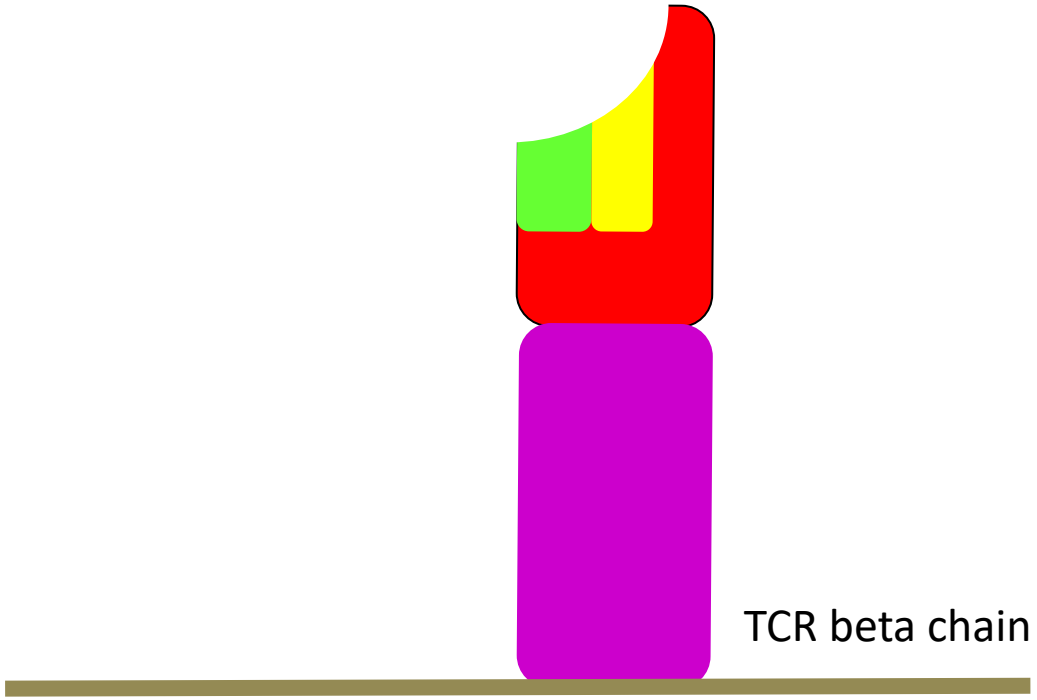
The T cell randomly picks one of each of the V and J segments

Generating T cell diversity

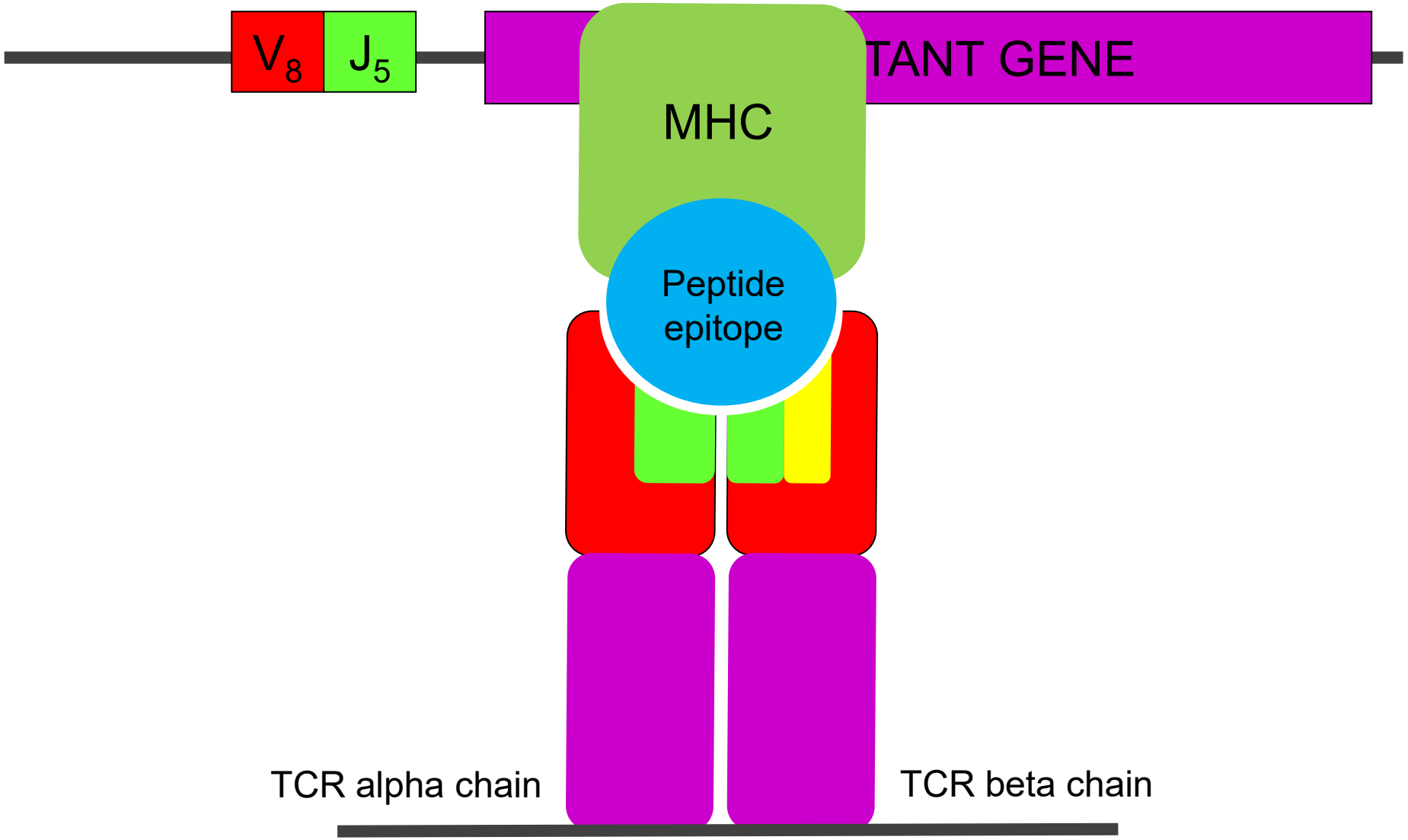


The particular V(D)J combination determines the shape of the antigen-binding site

TCR beta chain gene



TCR alpha chain gene



Positive selection

- During positive selection TCR is screened for reactivity against self MHC molecules
- Those T cells whose TCR ‘prefers’ MHC class I retain CD8; MHC class II = CD4
- T cells whose TCR doesn’t bind particularly well to MHC molecules fail to survive.
- MHC genotype will dictate this process
  - Some MHC-types might be better than others at selecting a wide repertoire
  - MHC heterozygosity is probably better than than homozygosity

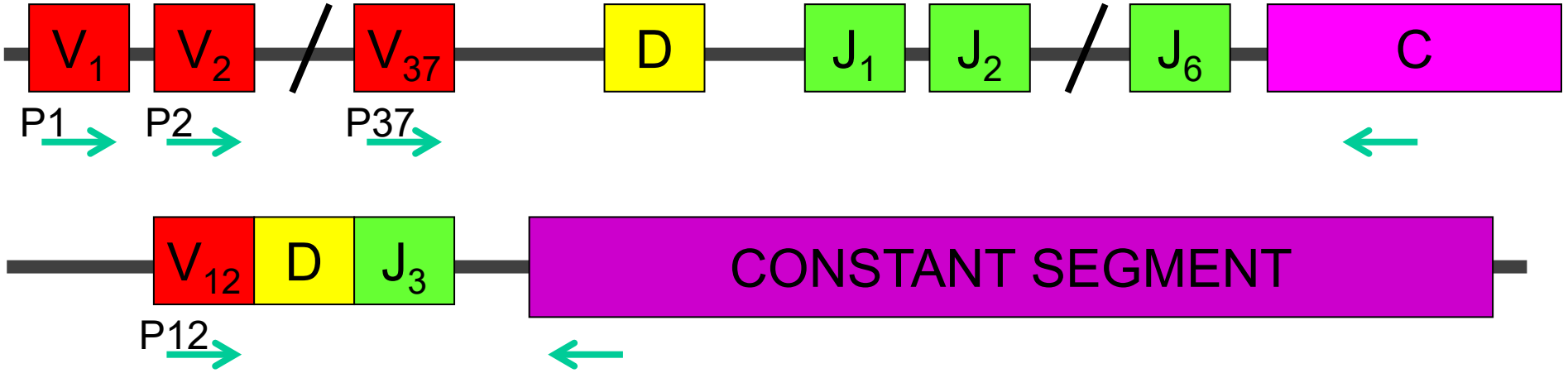
MHC influence on T cell selection

- Rottweilers seem to be poor responders to CPV and rabies vaccination – deficient repertoire ?
- They only have two MHC-types in the entire breed
  - DRB*006--DQA*005--DQB*007 (common in many breeds)
  - DRB*001--DQA*003--DQB*004 (only seen in Rottys)
    - We suspect that this is the problem one, if they are homozygous

MHC influence on T cell selection

- Cocker spaniels also have a very restricted MHC profile, which seems to increase susceptibility to hypersensitivity (autoimmune) reactions – dysfunctional repertoire ?
- They only have two MHC-types in the entire breed
  - DRB*006--DQA*005--DQB*007
  - DRB*006--DQA*005--DQB*020

TCR V*beta* repertoire analysis

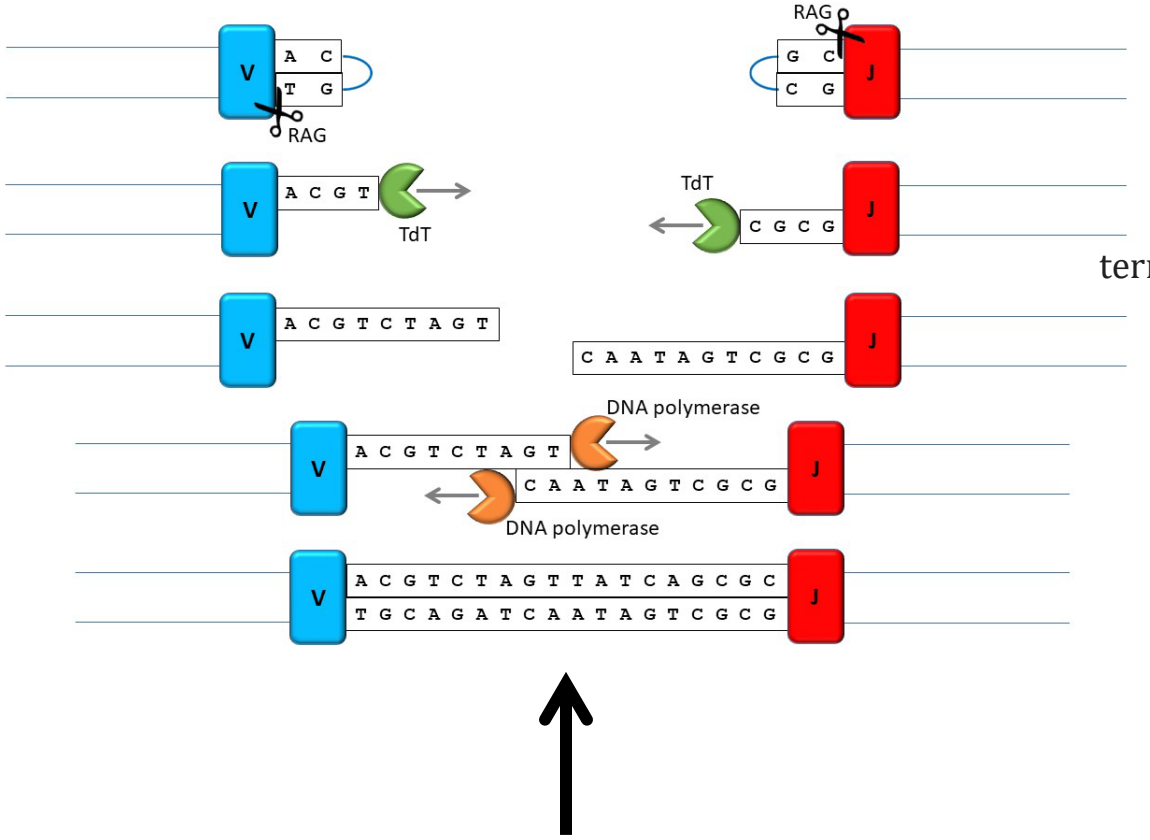


Diverse “healthy” repertoire

Potentially abnormal repertoire due to selection

Potentially abnormal repertoire in ageing / disease

VDJ Recombination generates further diversity when ‘recombination’ occurs

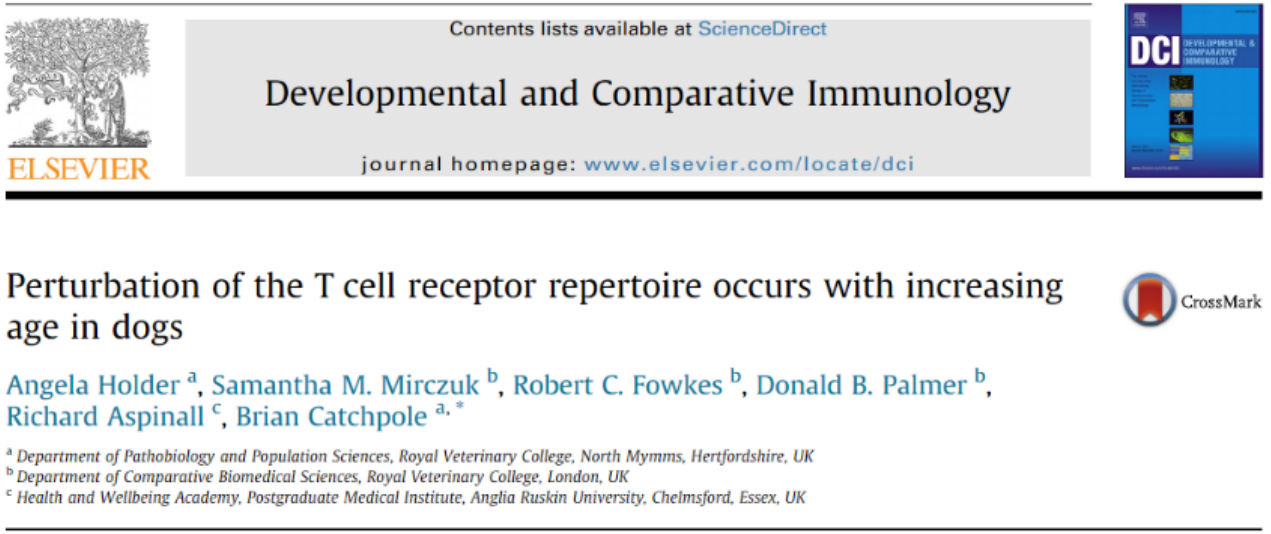


terminal deoxynucleotidyl transferase (TdT)

The size of this ‘infill’ (CDR3) is variable in different T cell clones within a Vbeta family

T cell repertoire analysis

- Forward primers for each of the canine V genes
- Reverse primer in Constant region



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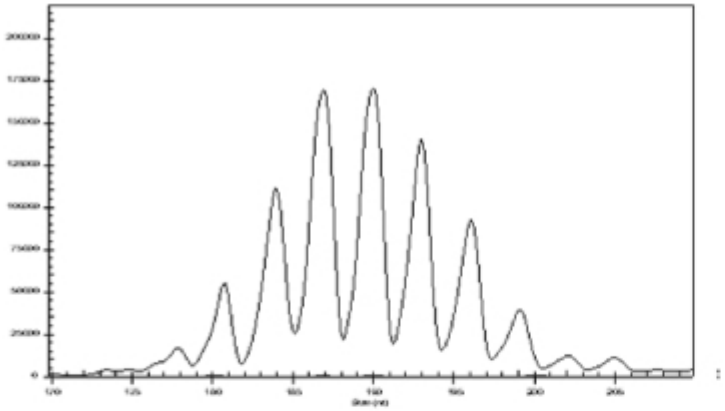
Perturbation of the T cell receptor repertoire occurs with increasing age in dogs

Angela Holder [a], Samantha M. Mirczuk [b], Robert C. Fowkes [b], Donald B. Palmer [b], Richard Aspinall [c], Brian Catchpole [a,*]

[a] Department of Pathobiology and Population Sciences, Royal Veterinary College, North Mymms, Hertfordshire, UK
[b] Department of Comparative Biomedical Sciences, Royal Veterinary College, London, UK
[c] Health and Wellbeing Academy, Postgraduate Medical Institute, Anglia Ruskin University, Chelmsford, Essex, UK

CDR3 spectratyping

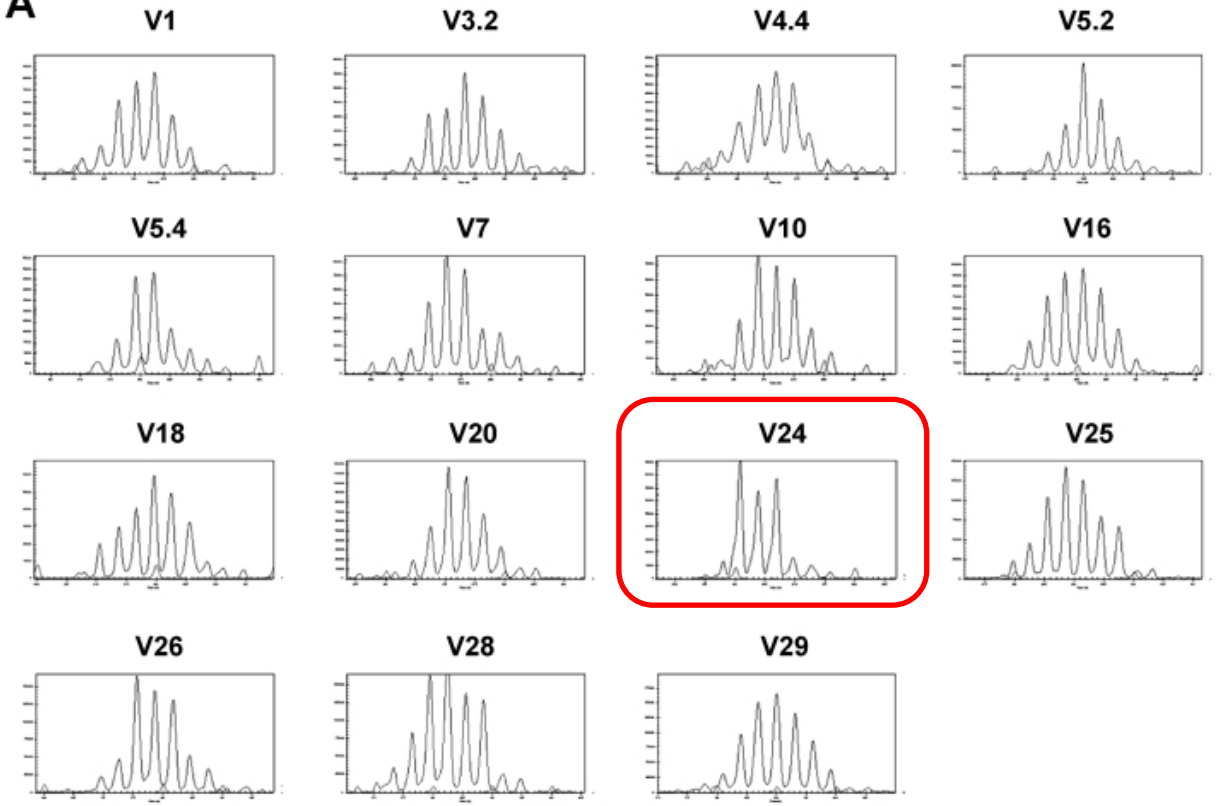


Normal
(Gaussian distribution)

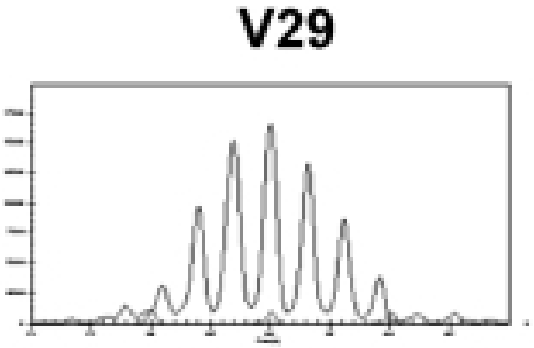
Skewed =
Clonal expansion of memory cells
and/or
Loss of naïve populations

YOUNG DOG

A

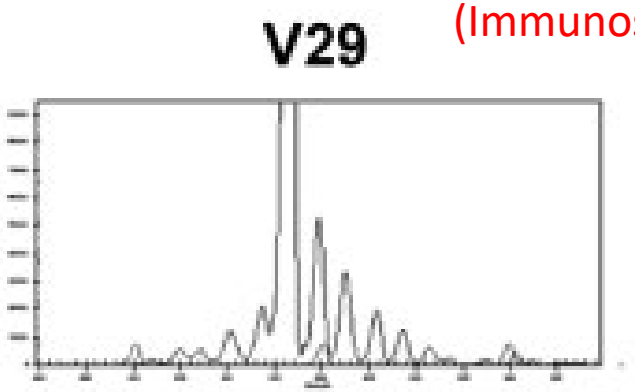


The occasional skewed profile might result from vaccination?



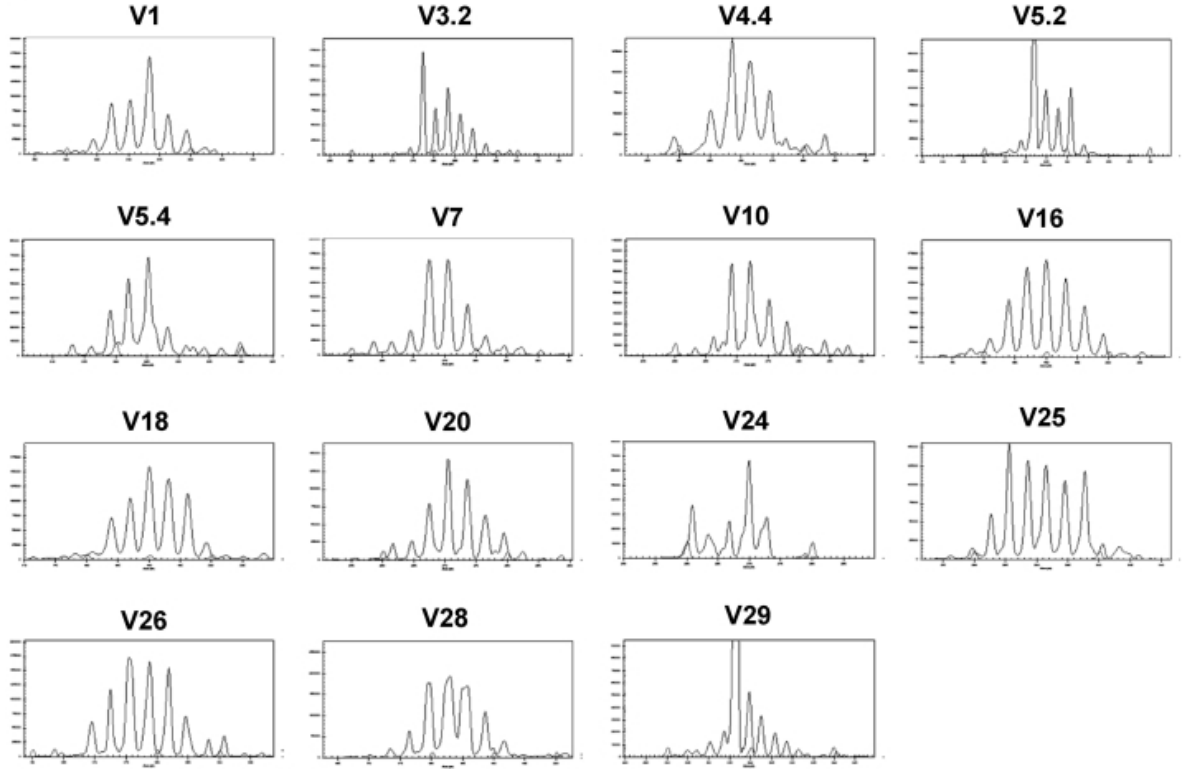
Polyclonal profile

Oligoclonal profile
(Immunosenescence?)



OLD DOG

B



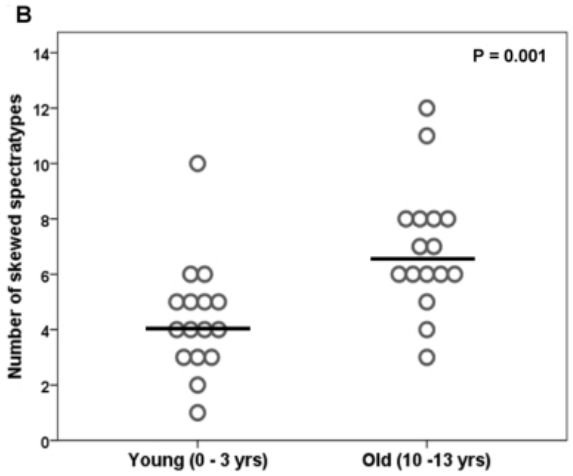
Increased clonality and reduced diversity in older dogs

Number of skewed spectratypes

A

TCRB family	Young Labradors (0 - 3 yrs)																Old Labradors (10 - 13 yrs)															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
V1		■									■			■	■				■	■								■	■			
V3.2					■															■	■	■	■		■					■	■	
V4.4	■	■		■						■	■						■	■	■	■	■	■	■	■		■				■	■	■
V5.2		■						■	■		■	■		■	■	■																
V5.4	■	■	■		■			■	■		■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
V7													■	■																		
V10	■		■	■		■			■				■	■											■				■	■		■
V16																		■	■	■	■											
V18														■																		
V20								■			■																					
V24	■	■	■	■		■	■		■	■		■		■	■	■																
V25													■					■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
V26			■	■				■	■		■			■																		
V28														■																		
V29	■						■																									
No. Skewed	5	5	4	4	3	2	1	5	6	4	5	3	6	10	3	4	6	12	8	8	6	4	6	7	6	5	3	11	6	8	8	7

B



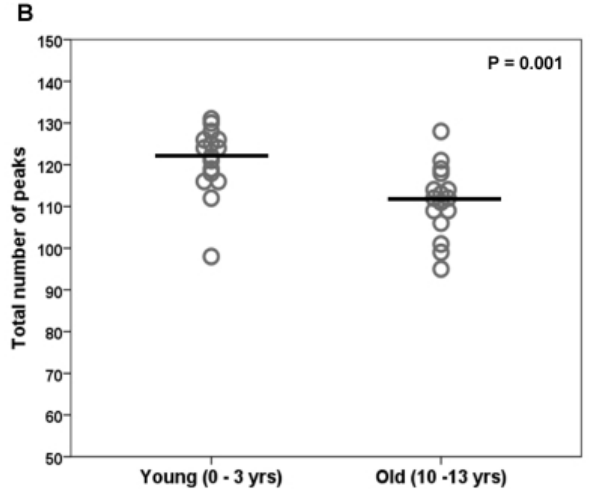
Total number of peaks

Normal = 8-10 peaks

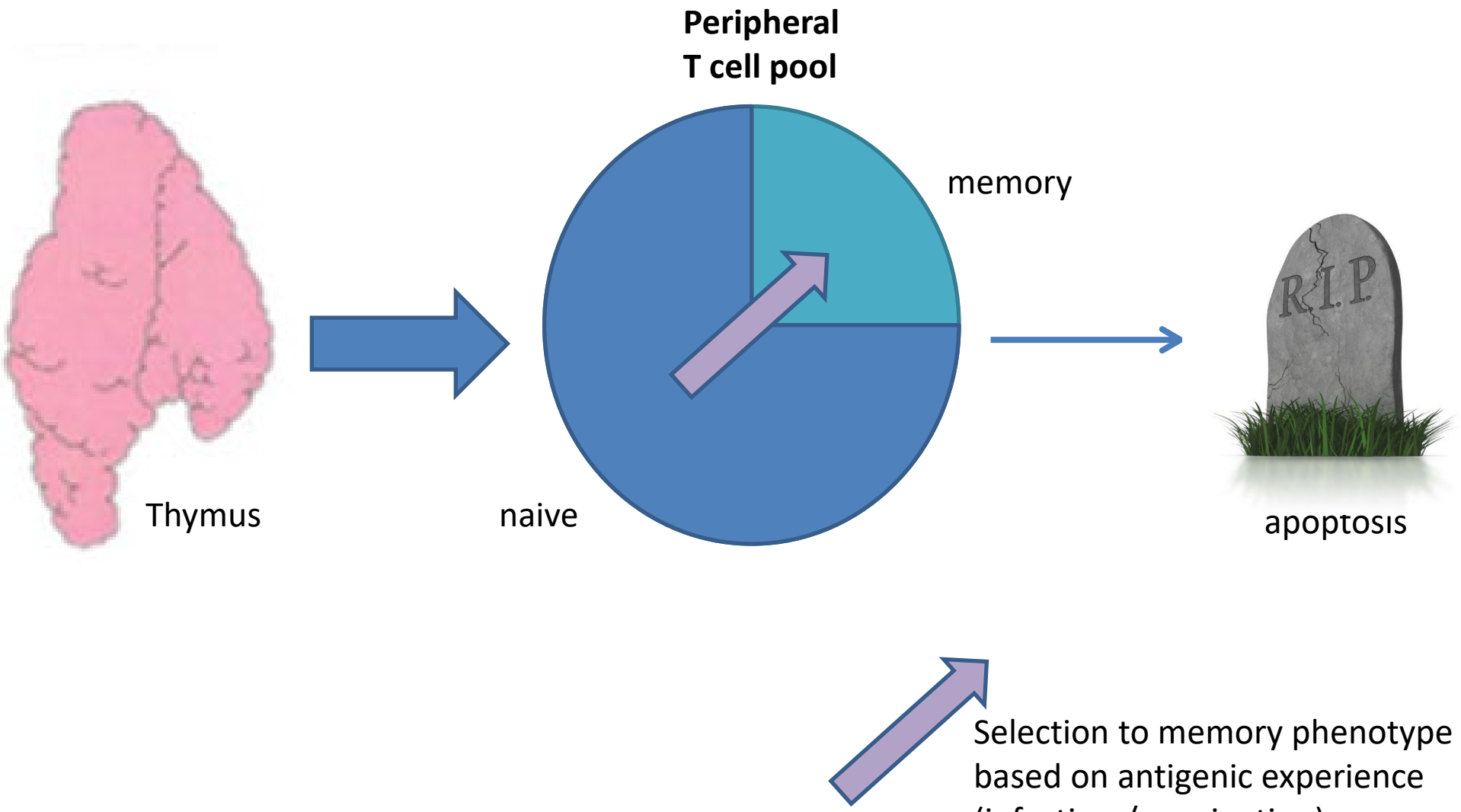
A

TCRB family	Young Labradors (0 - 3 yrs)																Old Labradors (10 - 13 yrs)															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
V1	8	7	8	8	8	10	10	7	7	8	8	8	7	7	8	9	8	6	7	7	5	8	8	6	7	8	8	7	10	6	8	8
V3.2	7	8	0	10	7	8	10	8	7	9	8	8	7	8	8	8	8	6	8	7	8	7	7	8	7	10	10	9	7	0	7	8
V4.4	7	6	9	9	7	8	9	7	8	8	7	7	6	10	9	10	9	8	8	9	8	7	8	9	8	6	8	7	7	8	9	7
V5.2	8	8	9	9	9	7	8	9	7	7	7	8	5	7	9	9	7	7	6	6	9	7	8	7	8	9	9	7	6	7	6	10
V5.4	8	6	7	8	7	8	7	7	5	8	5	8	4	6	6	7	5	4	3	4	6	7	6	6	6	6	7	7	5	6	6	5
V7	9	9	9	9	9	9	9	8	8	9	8	9	9	8	8	8	8	8	8	8	8	8	8	7	9	9	9	9	8	8	8	9
V10	9	9	8	9	9	7	9	7	7	8	8	9	5	6	8	8	8	6	7	5	8	8	7	9	7	9	7	7	7	8	3	8
V16	9	8	10	8	9	8	10	9	8	8	10	10	9	7	8	10	8	7	7	8	10	9	8	9	9	10	9	8	9	8	8	9
V18	8	10	8	10	9	9	9	8	8	9	10	9	9	6	9	10	8	8	8	8	8	7	7	7	10	10	10	6	9	7	9	10
V20	10	8	10	9	10	8	9	9	9	7	9	10	7	8	8	10	8	9	8	8	9	9	8	8	7	8	8	8	10	7	8	9
V24	7	6	6	8	8	8	8	8	6	7	8	7	0	8	6	7	8	4	7	6	5	5	6	7	6	8	7	5	5	5	0	6
V25	10	8	10	9	9	8	9	9	9	9	10	9	9	9	10	10	7	5	8	10	8	10	9	7	10	6	8	9	9	8	8	7
V26	8	8	9	7	8	9	8	7	7	10	8	8	6	9	7	8	7	6	7	8	8	8	7	8	8	7	10	8	7	7	6	8
V28	10	7	9	8	9	9	8	8	10	9	10	8	8	8	9	9	7	6	7	10	8	8	7	7	8	8	10	7	7	8	8	8
V29	6	8	6	7	8	8	7	7	6	7	8	9	7	8	8	8	6	5	7	5	6	6	8	6	8	7	8	5	7	6	7	7
Total	124	116	118	128	126	124	130	119	112	122	125	126	98	116	121	131	112	95	106	109	114	114	112	111	118	121	128	109	113	99	101	119

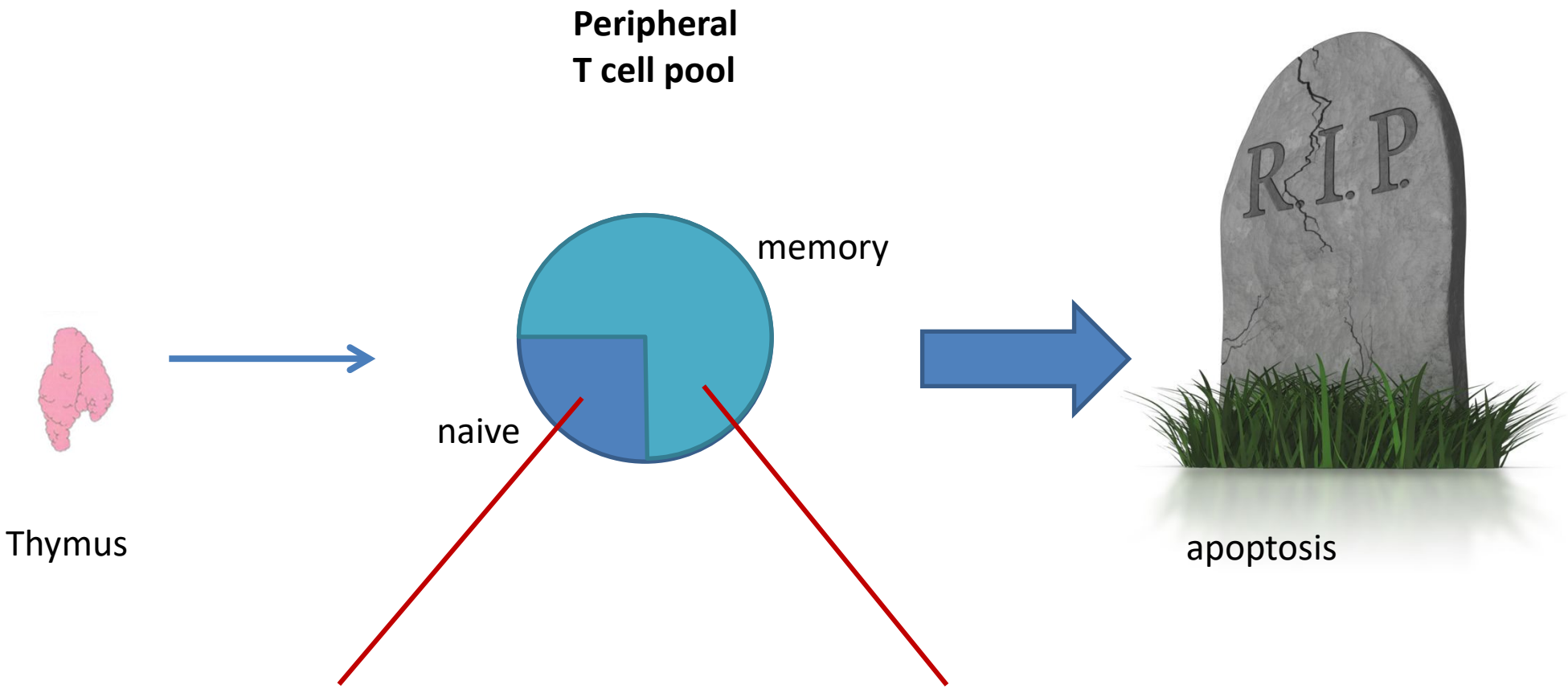
B



Healthy/juvenile immune system



Immunosenescence

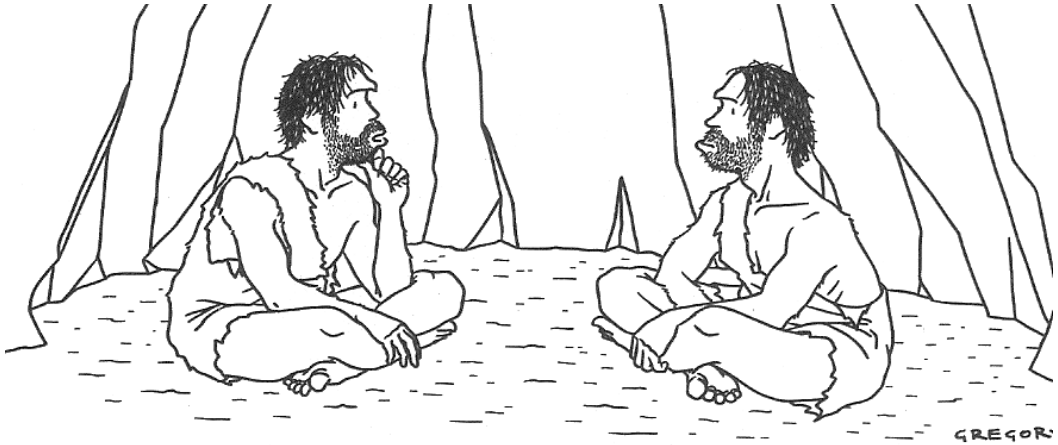


Summary

- Important developmental processes occur in the thymus
- The sj-TREC biomarker can be used to quantify RTEs in blood
- There is individual variation in thymic output throughout life
- Premature immunosenescence might occur in individual dogs depending on their genotype
- The T cell repertoire changes during the lifetime
- In old age there may be a disproportionate number of memory T cells and a relative deficiency in naïve T cells
- Older animals may maintain their ability to respond to pathogens against which they have previously been primed, but struggle to respond adequately to novel antigenic challenges

Immunosenescence

- In evolutionary terms, the immune system has to keep us alive long enough to reproduce and pass on our genes to the next generation
- It was never designed to keep us alive forever…



"Something's just not right—our air is clean, our water is pure, we all get plenty of exercise, everything we eat is organic and free-range, and yet nobody lives past thirty."