Discovery of the Thymus as a central immunological organ

1000
1000
[The Soul](#)
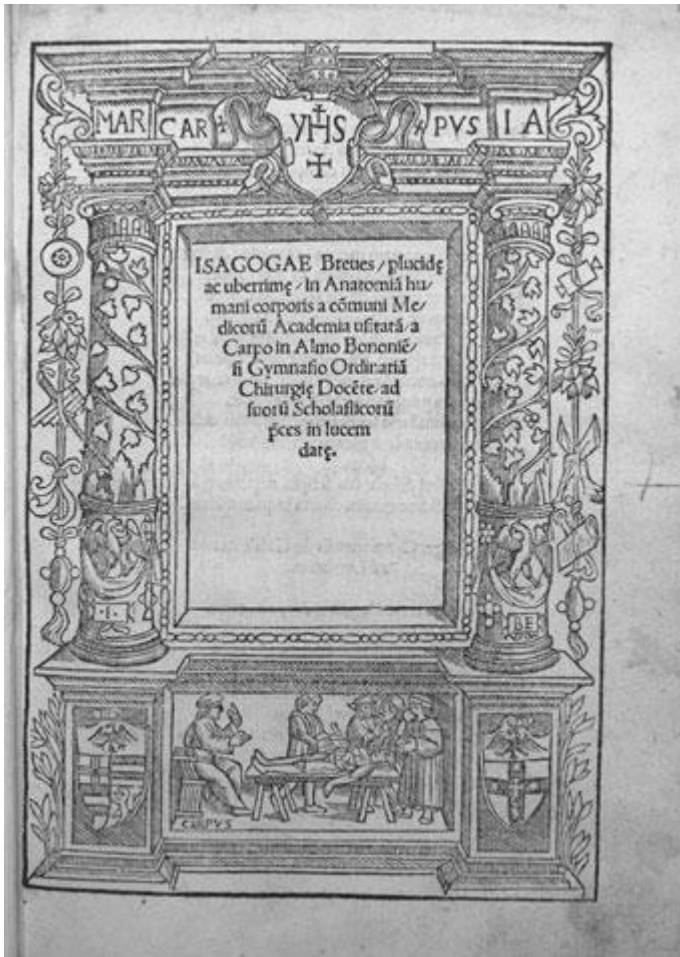

"The ancient Greeks, who performed sacrificial rites on very young and generally prepubertal animals, noted an extensive mass of tissue in the chest above the heart extending for some distance up into the neck and concluded that it must be the seat of the ´soul´." [Miller JAFP, Thymus 1:3-25, 1979](#)

Video: https://youtu.be/NnMZ0Uxol-M

1522
1522
[Isagoge](#)

Jacopo Berengario da Carpi in his *Isagoge breves* made the first description of the thymus

Bach J-F, Endeavour 2:154-160, 1978

1773
1773
Particles

William Hewson wrote 'The thymus gland we consider as being an appendage to the lymphatic glands, for the more perfectly and expeditiously forming the central particles of the blood of the foetus, and in the early part of life. We have proved that vast numbers of central particles made by the thymus and lymphatic glands are poured into the blood vessels through the thoracic duct and if we examine the blood attentively we see them floating in it.'

Cited by Doyle D, Brit H Haematol 133:375-381, 2006

1900
1900
Leukocytes

**Figure 1.** The only known photograph of John Beard.

In 1900, John Beard wrote "It has fallen to my lot to show that the first leukocytes arise in the thymus, from its epithelial cells, and that the thymus must be regarded as the parent source of all the lymphoid structures of the body…., so the original leukocytes, starting from their birth place in the thymus, have penetrated into almost every part of the

body, and have created new centres for growth, for increase, and for useful work for themselves and for the body" [Miller JFAP, Thymus 1:3-25, 1979](#)

1933
1933
[Mesenchyme](#)
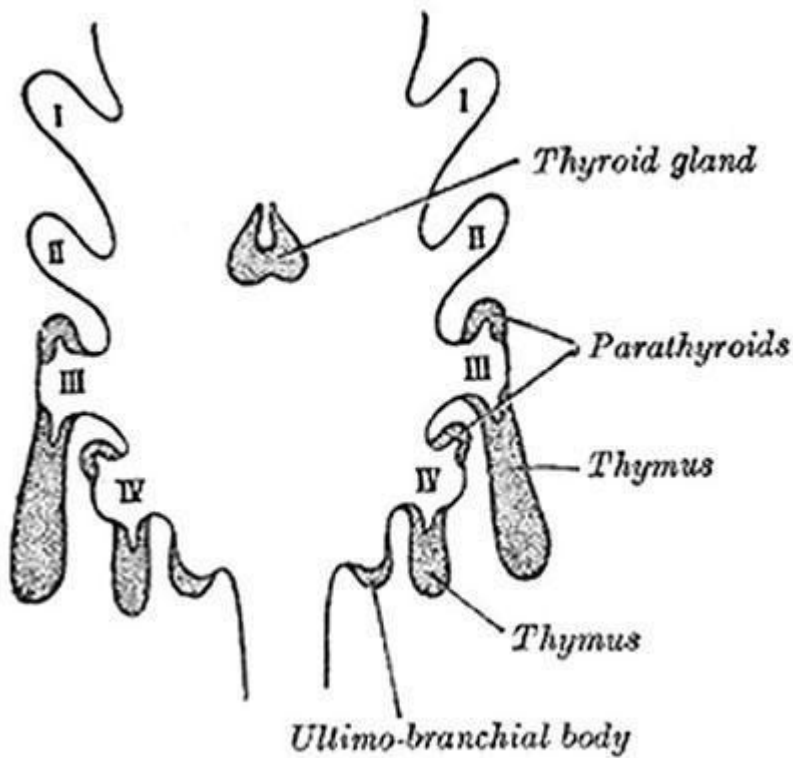
Weller reported that the thymus and the parathyroids are formed by the third and fourth endodermal pharyngeal pouches that migrate laterally in surrounding mesenchyme

[Pahwa et al., Thymus 1:27-58, 1979](#)

1955
1955
[Good's Syndrome](#)

Robert A Good (1922-2003)

Robert Good and Richard Varco described a new syndrome characterized by thymoma, lymphopenia, decreased serum gamma globulins and increased susceptibility to infections by encapsulated microorganisms, virus and fungi. The syndrome was known as Good´s syndrome

Lancet 75:245-71, 1955

1957
1957
Clonal selection theory

A Modification of Jerne's Theory of Antibody Production using the Concept of Clonal Selection
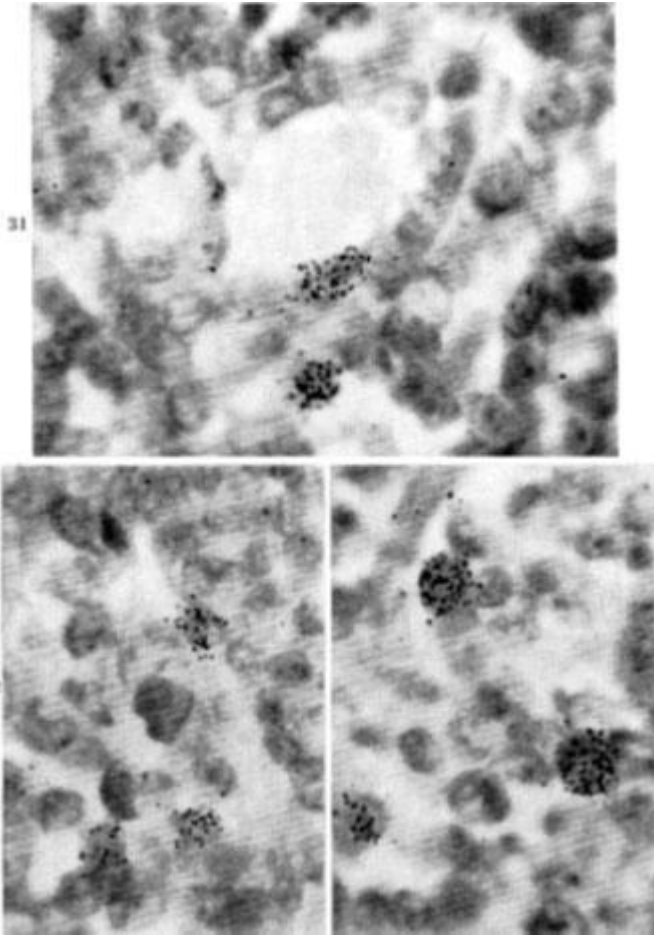
F.M. Burnet, M.D., Ph.D.

F. MacFarlane Burnet proposed in his "Clonal selection theory" that antigens are recognized by immunocompetent cells that either clonally expand to mount an immune response or are deleted resulting in tolerance. However, the identity of the immunocompetent cells was still unknown

Burnet, Austr J Sci 20:67-69, 1957

1960
1960
Lymphocytes

In the late 1950s and early 1960s, James Gowans and colleagues in Oxford University demonstrated that lymphocytes obtained from the thoracic duct of rats and mice, labeled with ^{3}H-thymidine and reinfused intravenously, were found in spleen and lymph nodes. Chronic drainage of thoracic duct resulted in depletion of small lymphocytes, unresponsiveness to different antigens and tolerance to skin homografts (allografts).

Gowans et al., Nature 196: 651-656, 1962; Gowans and Knight, Proc Roy Soc London B 159:257-282, 1964

1962
1962
Lymphocyte Differentiation

TABLE 1. PERIPHERAL LEUCOCYTE LEVELS OF 6-WEEK-OLD $(Ak \times T6) F_1$ HYBRID MICE THYMECTOMIZED AND SHAM-THYMECTOMIZED AT BIRTH

treatment	no. of mice in group	peripheral leucocytes per mm³			
		total	lymphocytes	neutrophils	eosinophils and monocytes
thymectomy at birth	33	5640 ± 320*	2880 ± 200	1970 ± 210	690 ± 60
sham-thymectomy at birth	22	8730 ± 260	6600 ± 720	1390 ± 140	700 ± 70
statistical significance of difference	—	$P < 0.001$	$P < 0.001$	not significant	not significant

* = $\pm$ standard error.

F. A P. Miller in a series of classical experiments demonstrated that neonatally thymectomized mice are lymphopenic and immunodeficient and proposed that the thymus is the lymphoid organ where small immunocompetent lymphocytes are differentiated.

Miller, Lancet 278:748-749, 1961; Miller, Nature 195: 138-1319, 1962; Miller, Proc Roy Soc London B 16:415-428, 1962

1962
Immune Responsiveness
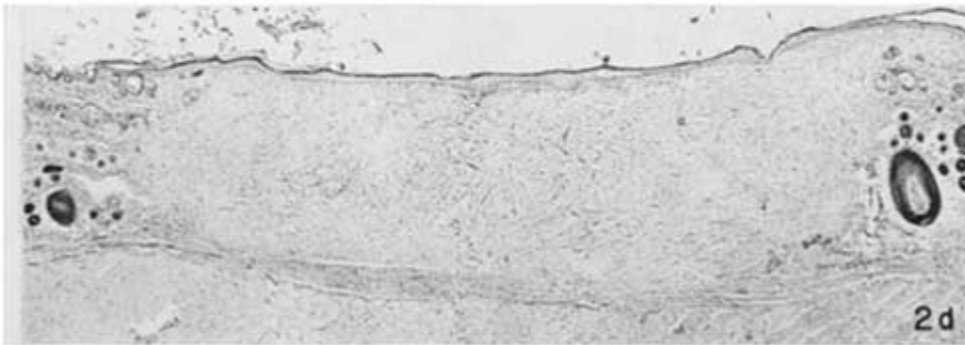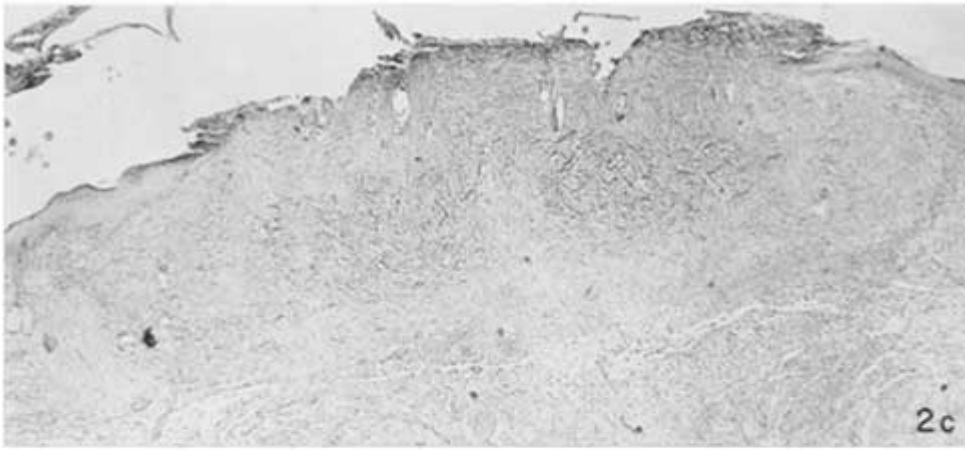
Other groups also demonstrated that the thymus is essential for the generation of immunocompetent lymphocytes and immune responsiveness

Jankovik et al., J Exp Med 116:159-176, 1962; Arnason et al., J Exp Med 116: 177-186, 1962; Waskman et al., J Exp Med 116: 187-205, 1962; Good et al., J Exp Med 116:773-795, 1962

1964
1964
Thy-1 Molecule

TABLE V

Cytolysis of Normal AKR Cells by Isoantisera Prepared in C57BL/6 Mice
(Differ in H-2 Allele) against AKR Cells*

C57BL/6 isoantisera against the following AKR cell types:	Potency of C57BL/6 isoantisera for cytolysis of the following normal AKR cell types:				
	Thymo-cytes	Lymph node cells	Spleen	Intraperitoneal lymphocytes	Marrow
Washed blood cells..........	290	36	62	23	2
Thymocytes................	450	61	82	9	2
Lymph nodes..............	<2	30	77	(20 % a.c.)‡	2
Spleen.....................	1.6	660	710	137	(21 % a.c.)
Marrow....................	<2	1.8	9§	(17 % a.c.)	2
Liver......................	<2	10.9	16.3§	(25 % a.c.)	2
L4946 leukemia	4	53	112	32	(21 % a.c.)
BW5147 leukemia...........	5.9	47	66	21	(12 % a.c.)
S775 leukemia..............	8.6	27	34	8.6	<2
T283 sarcoma..	<2	3.0	4.4§	<2	<2

* Each value represents the mean results of two determinations performed on different days.

‡ (20 % a.c.) means that the highest stained cell count was 20 *per cent above* the *control* level.

§ Potency for cytolysis of 60 ± 5 per cent of the cells; the remaining 40 per cent remained viable even at high concentrations of isoantiserum.

TABLE X

Absorption of C3HeB/Fe Isoantiserum against AKR Thymocytes by Packed Residues Derived from Homogenates of AKR and RF Tissues

Mouse strain	Tissue residue used for absorption	Absorptive capacity* relative to AKR thymus
		per cent
AKR	Thymus	100
	Brain	107
	Neonatal brain	1.6
	Appendix	3.3
	Lung	1.5
	Liver	0.9
	Skeletal muscle	0.8
	Kidney	0.6
	Testis	0.6
AKR	Hemisphere	152
	Pituitary	89
	Brain stem	76
	Cerebellum	57
	Spinal chord	54
	Olfactory bulb	41
	Sciatic nerve	29
RF	Thymus	71
	Brain	111
	Hemisphere	181
	Brain stem	79

* Calculated relative to absorption by an AKR thymic residue derived from the same wet weight of tissue. All results are the mean of two experiments.

Reif and Allen described the Thy-1 molecule (now CD90) as a cell differentiation marker in thymocytes, T cells and neurones.

J Exp Med 120: 413-433, 1964

1965
1965
B cells and T cells

Angelo M DiGeorge described the association of thymic aplasia, hypoparathyroidism and infection (DiGeorge´s syndrome). In the same paper Max D Cooper et al., proposed the existence of two types of immune responses: one mediated by lymphocytes generated in the Bursa of Fabricius in birds (B cells) and responsible for the production of antibodies, and the other mediated by lymphocytes differentiated in the thymus (T cells) and responsible for the cell-mediated immune responses

Cooper et al. J Pediatrics 67: 907-908, 1965
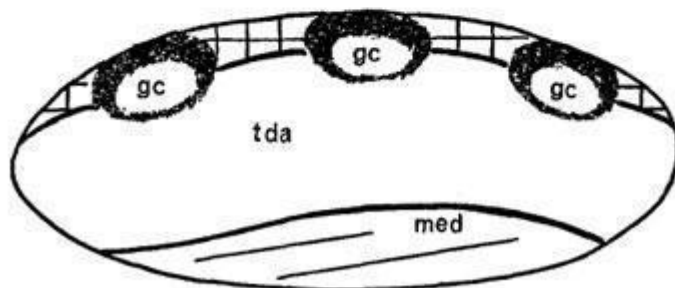
1966
1966
Histological Alterations
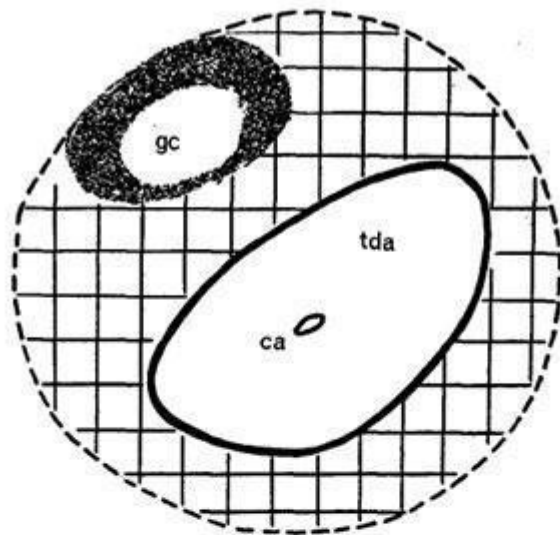
TEXT-FIG. 1. Diagram of a spleen follicle of a neonatally thymectomized mouse showing germinal center (gc) and thymus-dependent area (tda) surrounding central arteriole (ca).

TEXT-FIG. 2. Diagram of a lymph node of a neonatally thymectomized mouse showing follicles with germinal centers (gc) in outer cortex, the thymus-dependent area (tda) in the mid and deep cortex, and the medullary region (med).

Parrot et al., studied the histological alterations found in the spleen and lymph nodes of neonatally thymectomized mice and proposed that the lymphoid follicles surrounding the central arterioles of the spleen and the mid and deep cortical areas of the lymph nodes are thymic-dependent areas (tda).

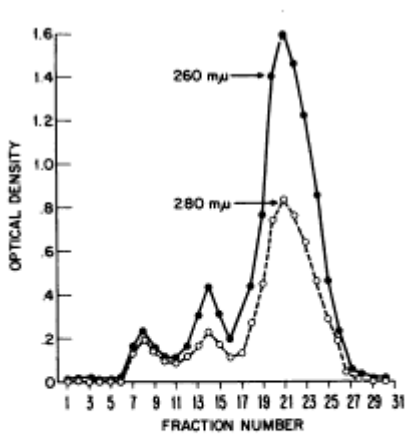J Exp Med 123:191-204, 1966

1966
Thymosin

FIG. 4.—Gel filtration of a soluble extract of a thymic fraction (*fraction 3*) on a Bio-Gel polyacrylamide P-10 column, 2 × 46 cm. The eluant was glass-distilled H₂O. Fractions were collected in 6-ml volumes; fractions *19* through *25* contained the major portion of the biological activity.
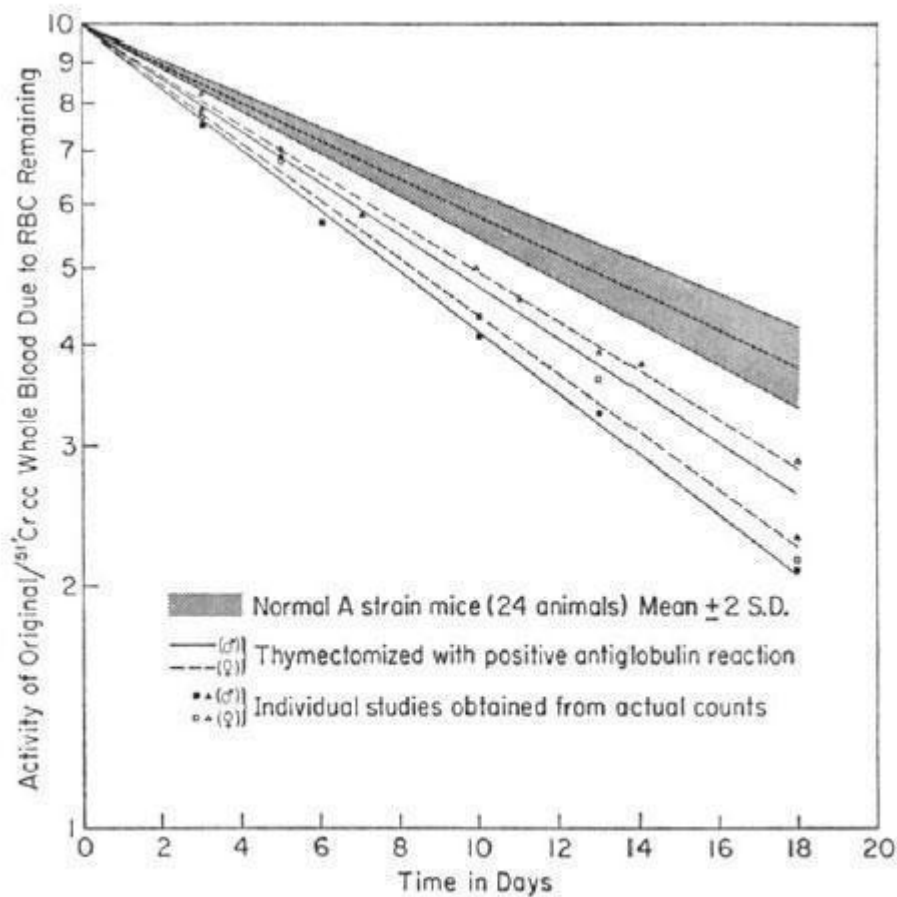
Goldstein et al., isolated from calf thymus a protein with lymphopoietic activity, that they termed Thymosin.

Proc Nat Acad Sci USA 56:1010, 1966

1967
1967
Autoimmune events

TEXT-FIG. 2. Chromium 51-labeled red blood cell survival study in normal and neonatally thymectomized A strain mice. The shaded area was obtained from the mean ± 2 SD of 24 normal animals. Four representative individual studies are shown.

Yunis et al., described the presence of autoimmune events in neonatally thymectomized mice suffering a wasting syndrome, characterized by failure to gain weight, hypothermia, diarrhea and early death.
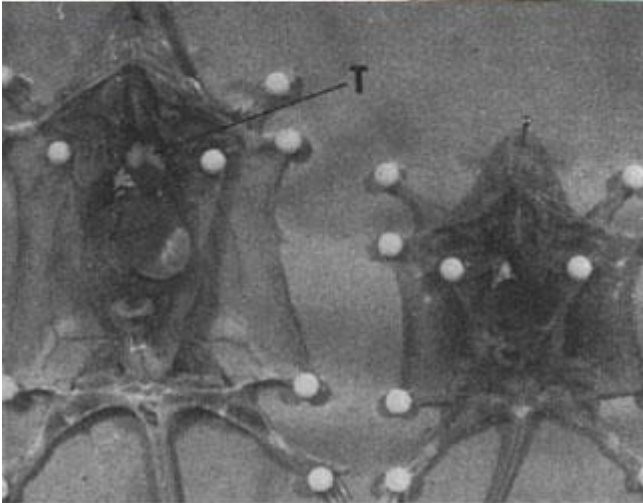
J Exp Med 125:947-966, 1967

1968
1968
Athymic

Fig. 1. Dissected normal (left) and homozygote nude (right) sibs. Note the absence of the thymus (T) from the latter.

Pantelouris showed that "nude" mice are athymic

Nature 217:370, 1968
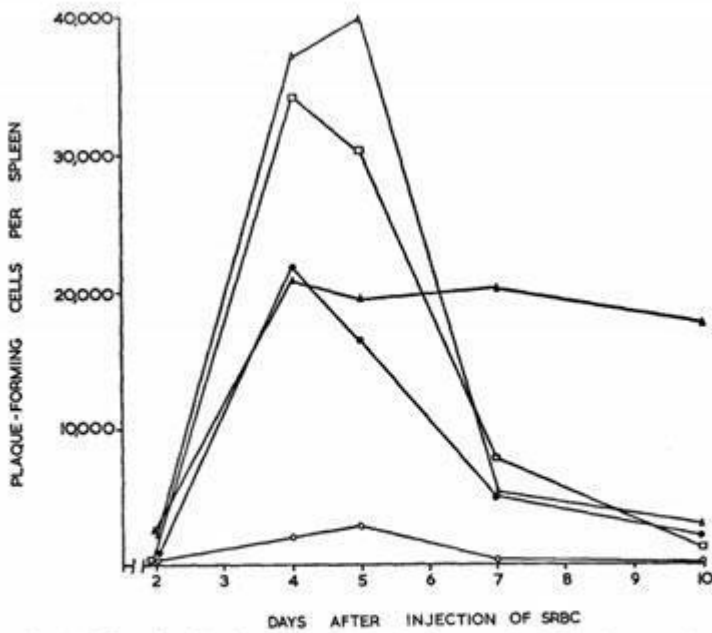
1968
Bone-marrow and Thymus

FIG. 1. PFC produced in the spleens of neonatally thymectomized CBA mice at various times after injection of SRBC and syngeneic thymus or thoracic duct cells. □——□, sham-operated controls given SRBC only; ○——○, neonatally thymectomized mice given SRBC only; ●——●, neonatally thymectomized mice given 10 million CBA thymus cells and SRBC; △——△, neonatally thymectomized mice given 50 million CBA thymus cells and SRBC; ▲——▲, neonatally thymectomized mice given 10 million thoracic duct cells and SRBC. The number of mice per point was 3-20 with an average of 7.

Miller and colleagues in Melbourne demonstrated that antibody-forming cells are bone marrow-derived but require the help of thymus-derived cells to produce antibodies
Mitchell and Miller, PNAS 59: 296-303, 1968; Miller and Mitchell J Exp Med 128: 801-819, 1968; Mitchell and Miller, J Exp Med 128:821-837, 1968
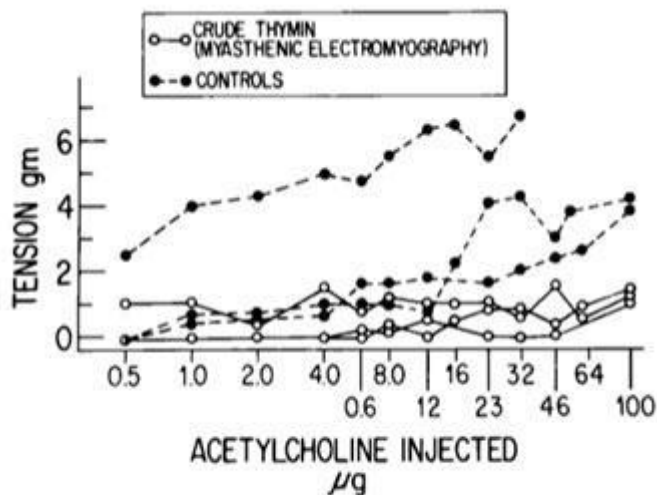
1971
1971
Thymin

FIGURE 6. Graphs showing tension responses of hemidiaphragm preparations from rats to varying doses of intravascularly injected acetylcholine. The dose-response curves in the rats made myasthenic with thymic extracts are impaired by comparison with the controls.
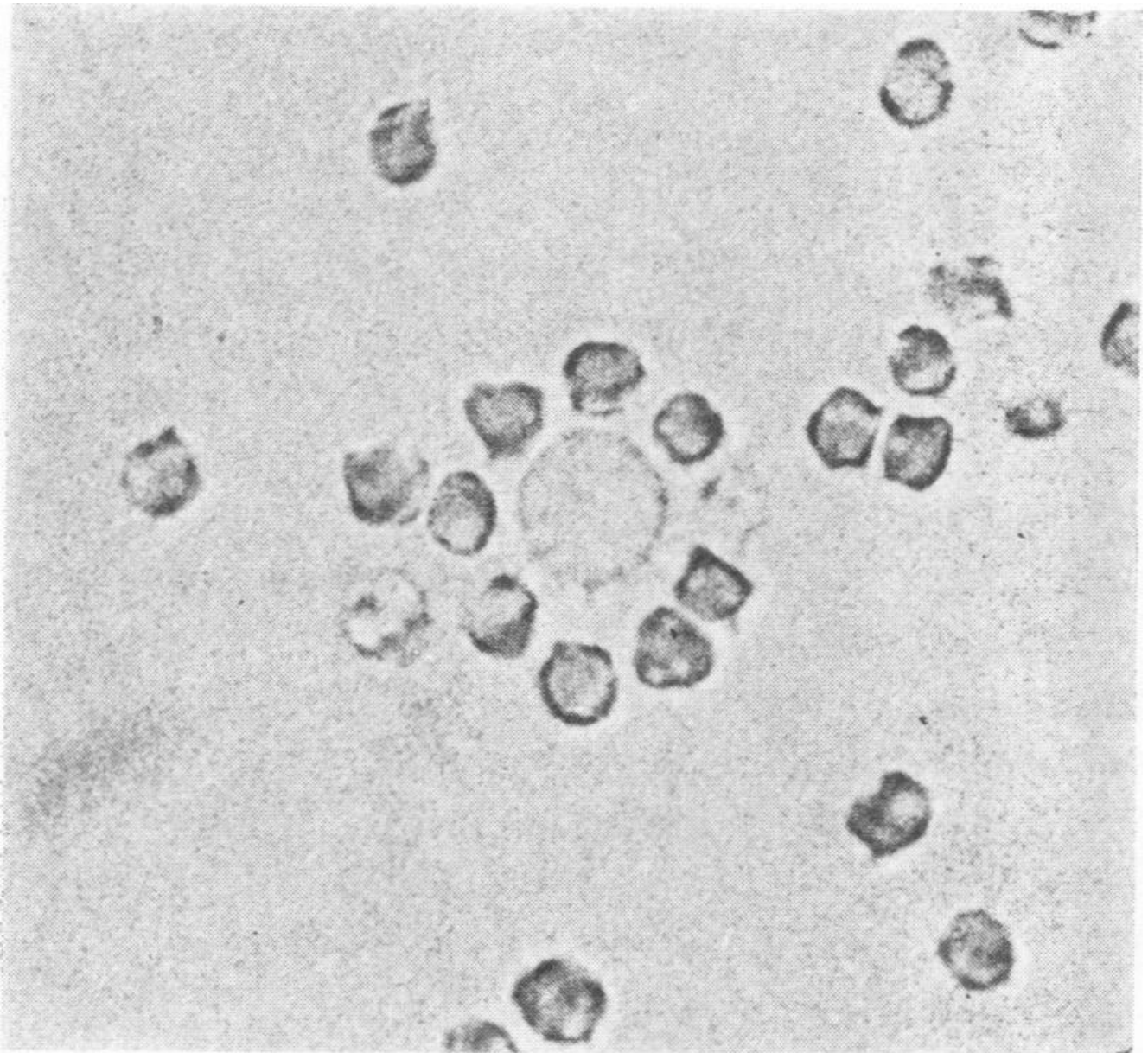
Goldstein and Manganaro reported the isolation from calf thymus of a factor that interferes with neuromuscular transmission and induces the expression of T cell maturation markers. This factor was called Thymin.

1972
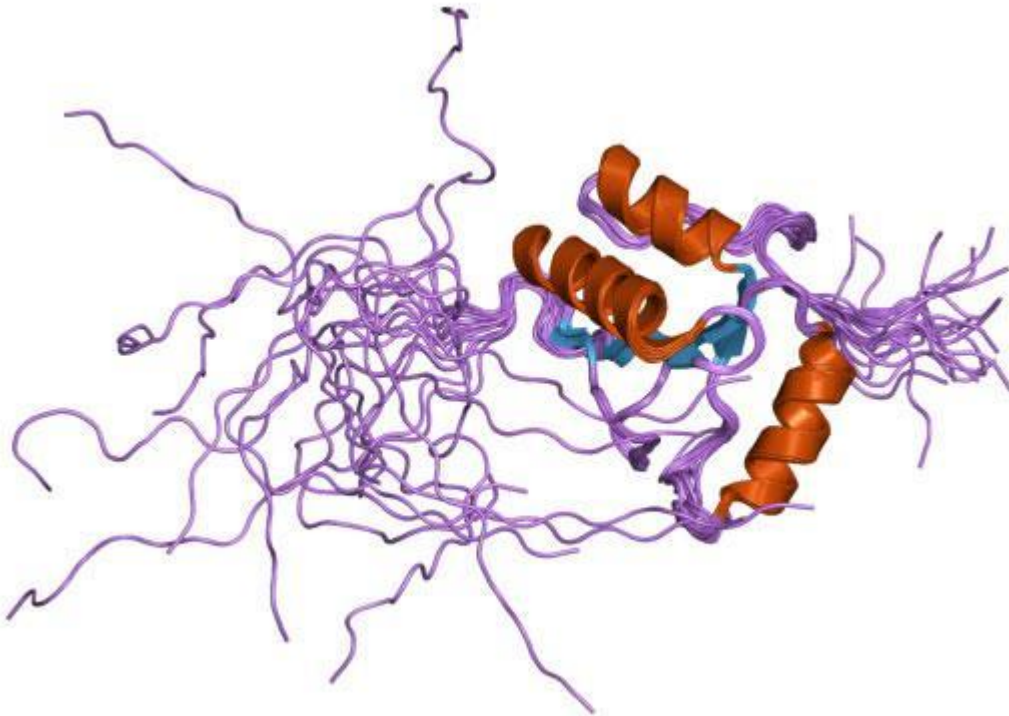1972
Facteur Thymique Sérique

Bach and Dardenne isolated the ´Facteur Thymique Sérique´ (FTS) that induces the expression of T cell antigens and restores the mitogen responsiveness in neonatally thymectomized mice.

Cell Immunol 3:1-10, 1972

1974
1974
TdT

Coleman et al., described the presence of terminal-deoxynucleotide-tranferase (TdT) in thymocytes.

Biochem Biophys Res Comm 58:1104-9, 1974

1976
1976
Ly Molecules

TABLE IX
T-Cell Subclasses*

Characteristics:	T_H	T_{CS}	T_E	References
Ly phenotype	1	23	123	2–4, 11–14, footnote 2
Helper activity (T-B, T-T)				
Primary response	+		?	2, 4, 11, Table I
Secondary response	+			12, Table II
Suppressor activity				
Primary response		?	?	
Secondary response (specific)		+ +	+	Tables II–IV
Allotype suppression		+		Footnote 2
Polyclonal induction		+		2
Killer activity				
Prekiller		+	?	4, 11
Killer-effector		+		4, 11–13
Delayed-type hypersensitivity	+			14

* T_{CS}, T cytotoxic/suppressor cell; T_E, T early appearing or immature cell; and T_H, T-helper cell.

In 1976, Cantor et al., reported that mouse thymocytes can be differentiated into four different subsets according to the expression of the Ly molecules: Ly123- (now CD4-CD8- or double negative, DN), Ly123+ (now CD4+CD8+ or double positive, DP), Ly1+ (now CD4+) and Ly2+3+ (now CD8+).

J Exp Med 143:1391-1401, 1976

1978
1978
MHC-restricted differentiating T cells

TABLE V

*Influence of Transplanted Irradiated Parental Thymus on Virus-Specific Cytotoxicity Generated in Adult Thymectomized with Syngeneic Bone Marrow Reconstituted F_1 Recipients**

Fetal liver or bone marrow donor	Thymus donor	Thymecto-mized recip-ient	Spleen cell to target cell ratio	^{51}Cr Release from vaccinia infected target cells‡		
				D2(d)	L(k)	MC57G(b)
Experiment 1:						
BALB/c × A (d × k|d) (Fetal liver)	None	BALB/c × A (d × k|d)	40:1 13:1	35 33	39 38	21 17
BALB/c × A (d × k|d)	BALB/c (d)	BALB/c × A (d × k|d)	40:1 13:1 4:1	85 § 76 63	40 39 39	18 16 15
C57BL/6 × A (b × k|d) (Bone marrow)	A (k|d)	C57BL/6 × A (b × k|d)	40:1 13:1 4:1	75 79 37	80 67 54	17 18 16
	Control:‡	C57BL/10	40:1 13:1	39 33	40 38	79 87

Zinkernagel et al., using thymic chimeras, demonstrated that

the thymus is the tissue where the MHC-restricted differentiating T cells are selected.

J Exp Med 147:882-896, 1978
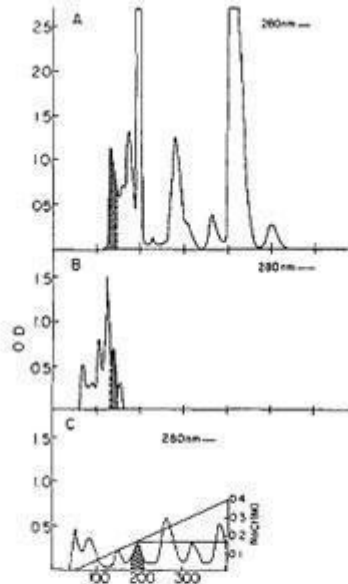
1978
Thymic Humoral Factor

FIG. 1.   Procedure for isolation of the active component of THF from calf thymus. A. The elution pattern of the dialyzate obtained from supernate of homogenized calf thymus on Sephadex G-10 columns. Shaded area represents activity eluted from the column in the void volume. B. The elution pattern of the active peak obtained from A on Sephadex G-25 superfine column. Shaded area represents activity eluted from this column. C. The elution pattern of the active peak obtained from B on DEAE Sephadex A-25 column. The column was equilibrated with 0.1 M NH₄HCO₃ pH 8.0 buffer and the material eluted with a linear gradient of NaCl. Shaded area represents activity eluted from the column.

Trainin and colleagues isolated a Thymic Humoral Factor (THF) able to induce immunocompetent cells in spleen from neonatally thymectomized mice.

J Exp Med 148: 71-83, 1978

1981
1981
T cell Hybridomas

Hybrid	Stimulus	IL-2 production				
		No Inhibitor	Hybridoma antibody inhibitor			
			11-5.2 ($I\text{-}A^b$)	10-3.6 ($I\text{-}A^{k,u}$)	MK-D6 ($I\text{-}A^d$)	MK-S4 ($I\text{-}A^u$)
AO-40.10	B10.A + OVA	+	−	−	+	+
	B10	+	+	+	+	+
	B6D2F$_1$	+	+	+	+	+
	(B10.A × D2)F$_1$ + OVA	+	−	−	+	+
AODK-1.16	B10.D2 + KLH	+	+	+	+	+
	B6D2F$_1$ + KLH	+	+	+	+	+
	(B10.A × D2)F$_1$ + KLH	+	+	+	+	+
AODK-10.4	B10.D2 + KLH	+	+	+	−	+
	B6D2F$_1$ + KLH	+	+	+	~	+
	(B10.A × D2)F$_1$ + KLH	+	+	+	−	+
AOFK-11.11.1	B10.A + OVA	+	−	−	+	+
	B10.M + KLH	+	+	−	+	−
	B10	+	+	+	+	+
	(B10 × B10.M)F$_1$	+	+	+	+	+
	(B10.A × B10.LG)F$_1$ + OVA	+	−	−	+	+

All SN were tested at 80% with HT-2 cells. Chicken OVA was used at 1,250 µg/ml. KLH was used at 1,000 µg/ml. Hybridoma antibodies were added to irradiated stimulator cells and antigen 1 h before the addition of the hybridoma cells. Antibodies were added in the form of 1% ascitic fluid taken from hybridoma-containing mice.

Kappler et al., generated antigen-specific, H-2-restricted, T cell hybridomas that recognize both antigen and H-2 using a single receptor.

J Exp Med 153:1198-1214, 1981

1983
1983
Monoclonal Clonotypic Antibodies

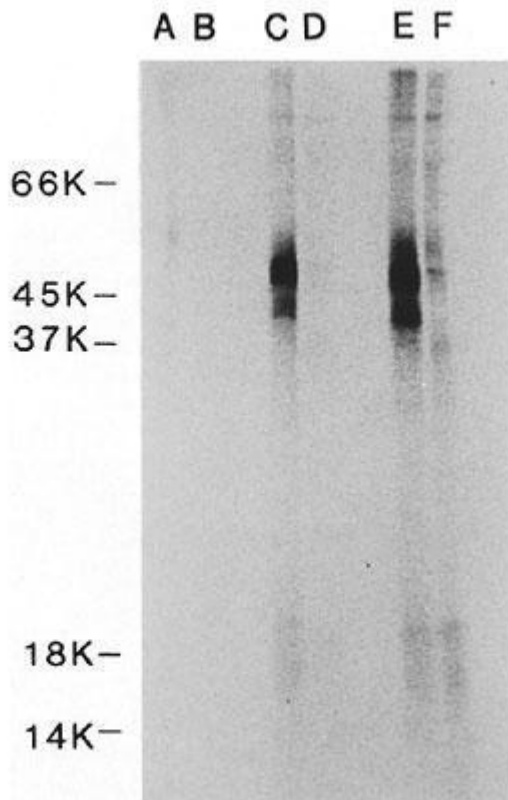FIG. 4. SDS-PAGE of immunoprecipitates from T cell clones CT8ᵢᵢᵢ and CT8ᵢᵥ. A: anti-T6 (CT8ᵢᵢᵢ); B: anti-T6 (CT8ᵢᵥ); C: anti-Tiₗₐ (CT8ᵢᵢᵢ); D: anti-Tiₗₐ (CT8ᵢᵥ); E: anti-Tiₗᵦ (CT8ᵢᵢᵢ); F: anti-Tiₗᵦ (CT8ᵢᵥ).

Reinherz and his colleagues ([J Exp Med 157:705-719, 1983](#)) using human T cell clones, and Marrack and colleagues ([J Exp Med 157: 1149-1169, 1983](#)) using mouse T cell hybridomas obtained monoclonal clonotypic antibodies that identified on the T cell membrane a heterodimer of 90KD associated with the T3 molecule (now CD3).
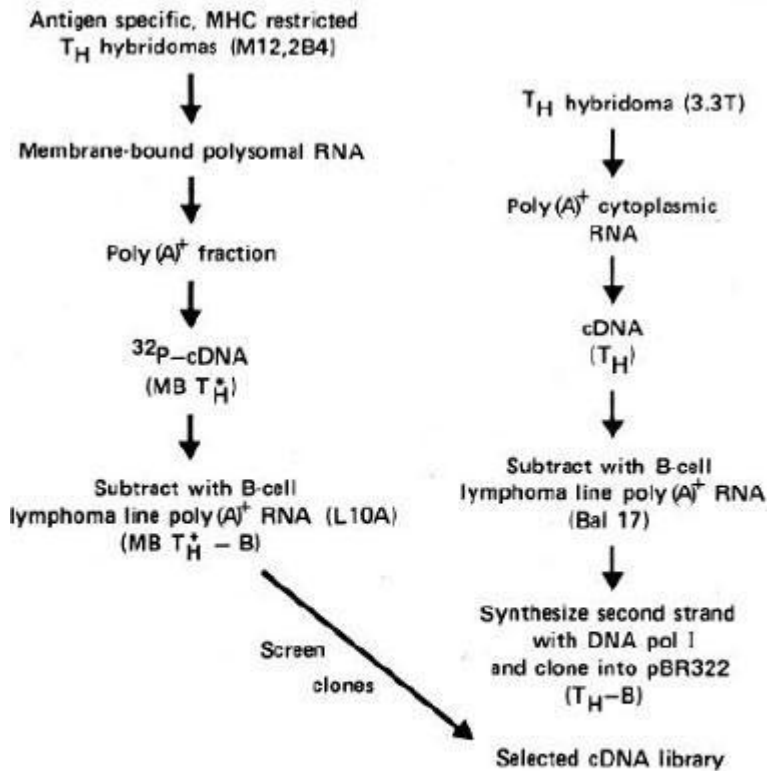
1984
1984
[T cell Receptor](#)

Fig. 2 Strategy of clone isolation. ^{32}P-labelled cDNA was synthesized from membrane-bound polysomal RNA of T_H hybridomas and subtracted with B-cell mRNA (L10A). These probes were then used to screen a selected cDNA library ($T_H - B$) constructed as described[30] from another T_H hybridoma/B cell combination.

Tak Mak and colleagues using differential screening (Yanagi et al., Nature 308: 145-149) and Mark Davis and colleagues using cDNA substractive hybridization (Hedrick et al., Nature 308: 149-153, 1984; Hedrick et al., Nature 308: 153-158, 1984), independently were able to clone the genes of the T cell Receptor (TCR), elucidate its basic structure, and demonstrate that the TCR genes, like the Ig genes, rearrange at the DNA level to generate the repertoire diversity.

1988
1988
Thymocytes Selection

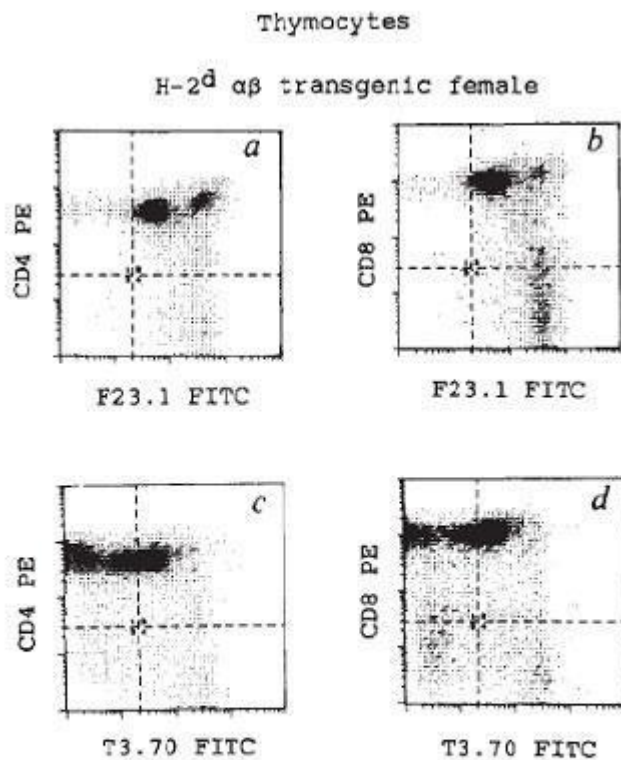Thymocytes

H-2^d $\alpha\beta$ transgenic female

Fig. 6 Staining of thymocytes from female $\alpha\beta$ transgenic H-2^d mice by F23.1, T3.70, CD4 and CD8 antibodies. Thymocytes (yield 7×10^7) were double stained with F23.1 and CD4 or CD8 (*a* and *b*) and with T3.70 and CD4 or CD8 (*c* and *d*).

In the late 1980s and early 1990s many groups showed that thymocytes are subject to a positive and negative selection during their intrathymus differentiation and maturation process. Among these groups, von Boehmer and colleagues (Teh et al., Nature 335:229-235, 1988) using TCR transgenic mice demonstrated that thymocytes undergo positive selection in the cortex of the thymus by binding of the TCR with the MHC expressed by the cortical epithelial cells. Also, Marrack and Kappler and their colleagues showed that once thymocytes migrate into the thymus medulla, they undergo a negative selection process leading to apoptosis and tolerance to specific antigens (Kappler et al., Cell 49:273-280, 1987; Kappler et al., Nature 332:35-40, 1988).

1997
1997
Autoimmune Regulator (AIRE) Gene
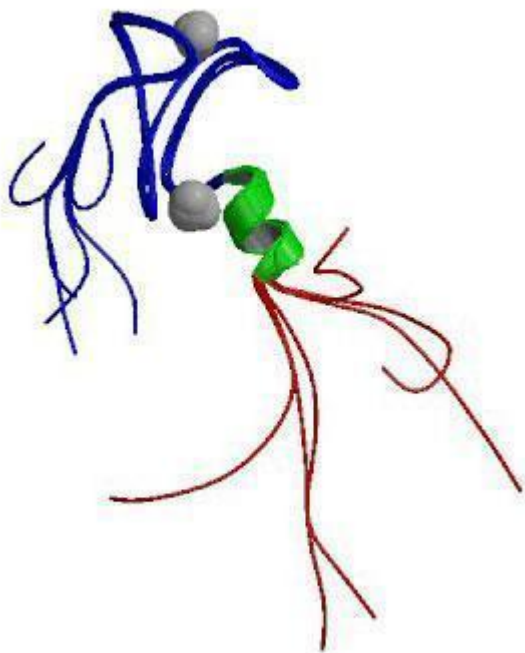
Mutations in the autoimmune regulator (AIRE) gene were identified as responsible for autosomal recessive Autoimmune Polyendocrinopathy-Candidiasis-Ectodermal Dystrophy (APECED) syndrome ([Nature Genetics 17:399-403, 1997](#)), also called Autoimmune Polyglandular syndrome type 1 (APS-1), and the human Autoimmune Regulator (AIRE) gene was mapped and cloned ([Nagamine et al., Nature Genetics 17:393-398, 1997](#)).
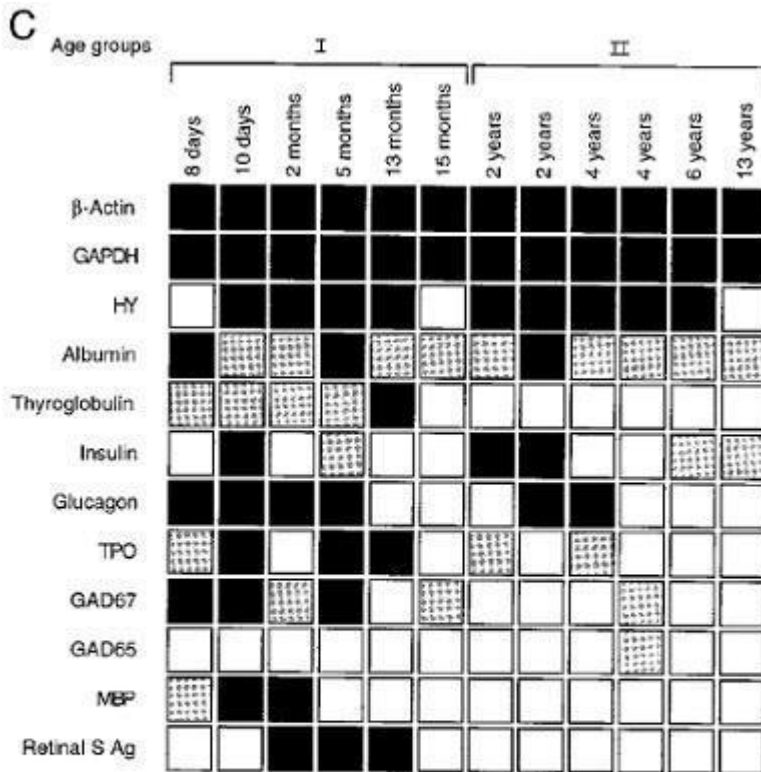
1998
1998
[Self-antigens Transcripts](#)

C

Age groups

	I						II					
	8 days	10 days	2 months	5 months	13 months	15 months	2 years	2 years	4 years	4 years	6 years	13 years
β-Actin												
GAPDH												
HY												
Albumin												
Thyroglobulin												
Insulin												
Glucagon												
TPO												
GAD67												
GAD65												
MBP												
Retinal S Ag												

FIGURE 3. Expression of peripheral Ag transcripts in thymic glands. *A*, Southern blots of amplification products of control and peripheral self-Ags from a panel of thymus samples. +, Control tissue, i.e., liver for albumin; thyroid for Tg and TPO; pancreas for insulin, glucagon, GAD65, and GAD67; brain for GAD65, GAD67, and MBP; and rat retina for Ret S Ag.
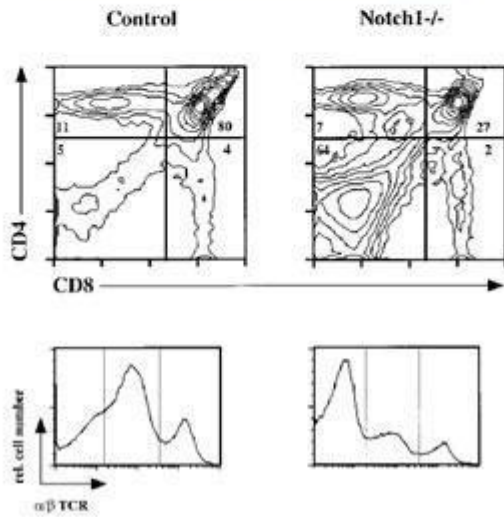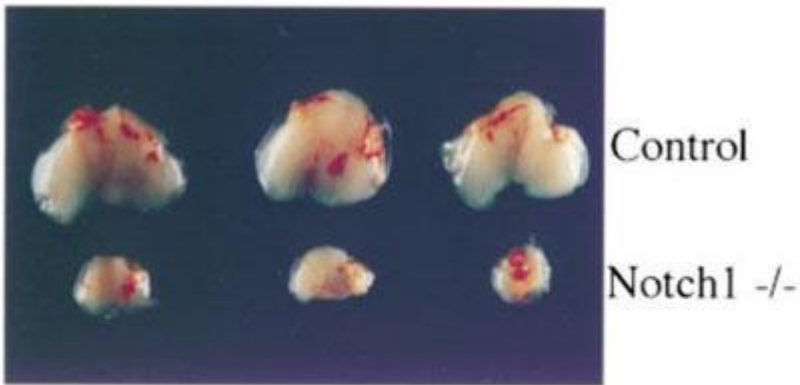
Sospedra et al., demonstrated the presence of a broad range of self-antigens transcripts in human thymus.
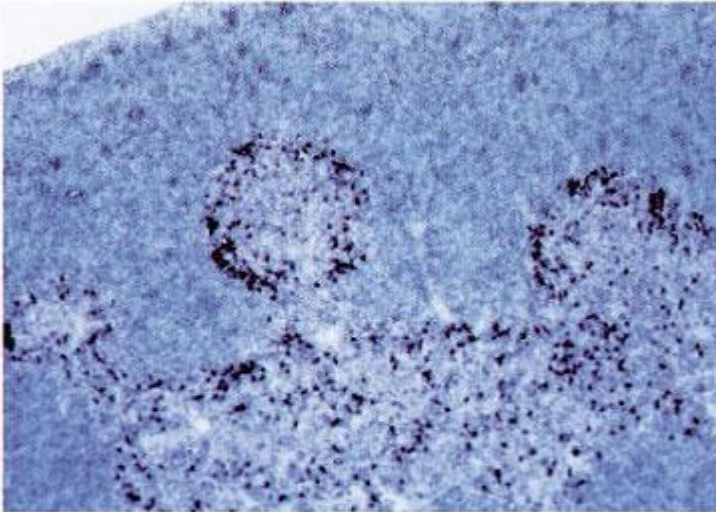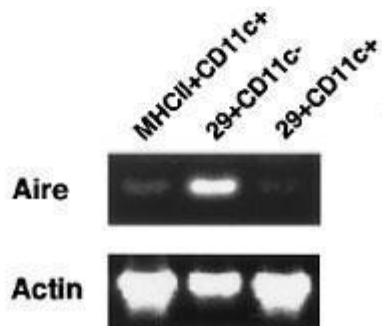
J Immunol 161:5918-5929, 1998

1999
1999
Notch1 Signals

Radtke et al., ([Immunity 10: 547-558, 1999](#)) and Pui et al. ([Immunity 11:299-308, 1999](#)) demonstrated the crucial role of Notch1 signals in the early T lineage determination.

2000
2000
[mTECs](#)

A

B

MHCII+CD11c+ 29+CD11c- 29+CD11c+

Aire

Actin

Zuklys et al., demonstrated that AIRE expression is restricted to medullary thymic epithelial cells (mTECs) and thymic dendritic cells and participates in the process of negative selection.

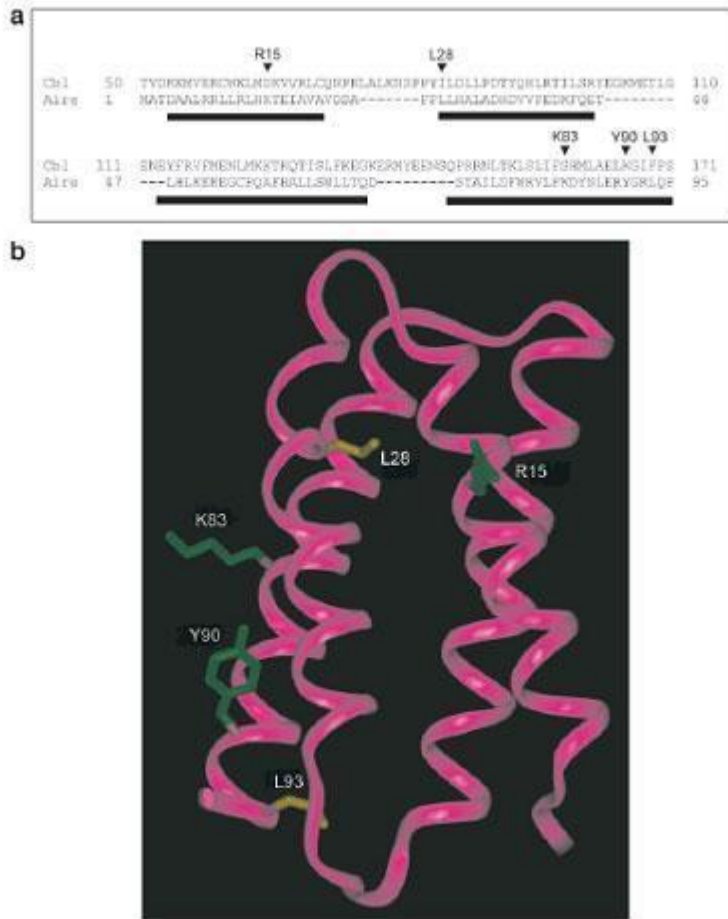J Immunol 165:1976-1983, 2000

2000
CREB-binding Protein

FIG. 5. **Molecular model of the HSR domain showing a four-helix bundle.** *A*, amino acid sequence alignment of amino acids 50–171 of CBL and amino acids 1–96 of AIRE. *Black bars* denote α-helices. Residues 15, 28, 83, 90, and 93 in the sequence of AIRE are indicated. These are the residues mutated in the disease-causing mutations R15L, L28P, K83E, Y90C, and L93R. *B*, three-dimensional model of the HSR domain. Side chains in *yellow* and *green* point into the inside of or outward from the structure, respectively. Same residues as in *A* are marked.

Pitkanen et al., showed that AIRE has transcriptional transactivator properties and interacts with the coactivator CREB-binding protein.

J Biol Chem 275:16802-16809, 2000

2001
2001
Tissue-restricted Antigens

Table 1. Summary of the cell type–specific pattern of promiscuous gene expression

	SAP	CRP	Complement C5	α-fetoprotein	Albumin	Haptoglobin	α1-antitrypsin	PLP	MOG	S100β	GAD67*	GAD65*	Somatostatin	Trypsin2	Elastase	Insulin*	Tyrosinase	Gp100	P1A	Thyroglobulin	Lactalbumin	α1-crystallin	Retinal S antigen	NAChRα1	IFABP	Amylase1
cTECs	–	–/+	+	–	–	–	–	+	–	–	–	–/+	–/+	+	–	–	+	–/+	+	–/+	+	+	–	–/+	+
mTECs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DCs	–	–	+	–	–	–	–/+	–	+	–	–	–/+	–	–/+	–	–	+	–/+	–	–	+	–	–	–	–
Macrophages	–	–	–/+	–	–	–	–/+	–	+	–	–	–	–	+	–	–	+	–	–	–/+	–/+	+	–	–/+

Genes are grouped according to their derivation from the organs of C57BL/6 mice. Note that all genes were expressed in mTECs. For designation of signal strength see Fig. 1. –, no signal ; –/+, no or weak signal in duplicate analysis; +, reproducible signal; nAChRα1, nicotinic acetylcholine receptor α1; gp100, melanosomal protein silver–Pmel17–gp87; iFABP, intestinal fatty acid binding protein; MOG, myelin oligodendrocyte glycoprotein. *Genes were also expressed by mTECs from NOD mice.

Derbinski et al., demonstrated that mTECs exhibit a

promiscuous expression of tissue-restricted antigens (TRAs).

Nature Immunol 2:1032-1039, 2001
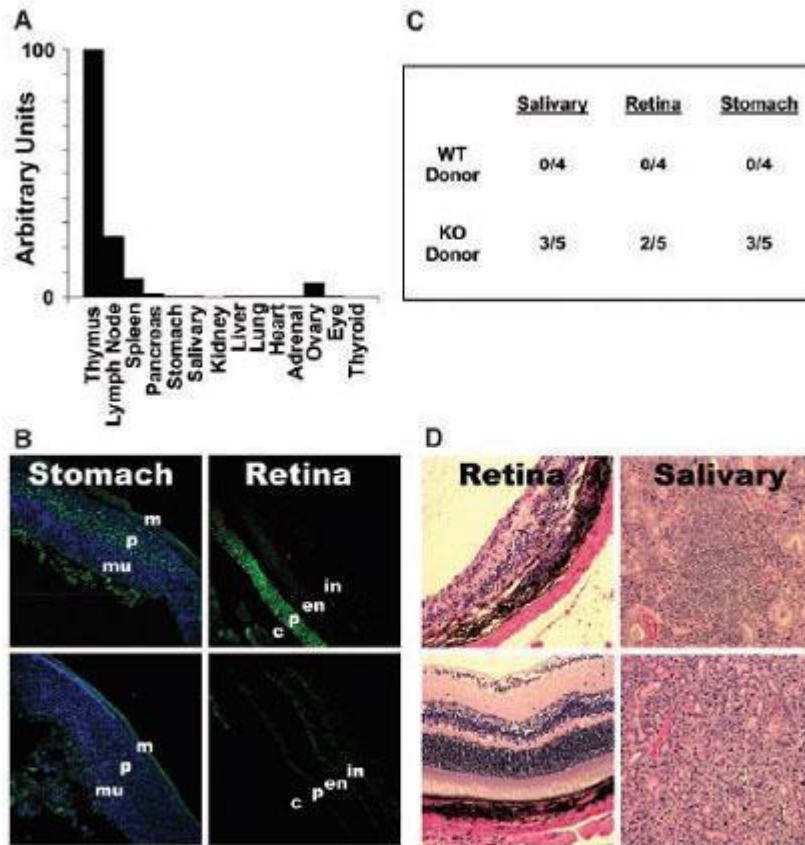
2002
2002
Aire Regulates

Fig. 4. The thymus is critical for aire-associated autoimmunity. (A) Aire is expressed predominantly in the thymus. Shown is relative expression of aire by quantitative real-time PCR using Taqman primers and probes on cDNA prepared from various whole organs from a 6-week-old B6 mouse. Normalization of cDNA content was done on cyclophilin; numbers represent a ratio of relative aire expression to cyclophilin expression for each tissue. For tissues other than thymus, lymph node,
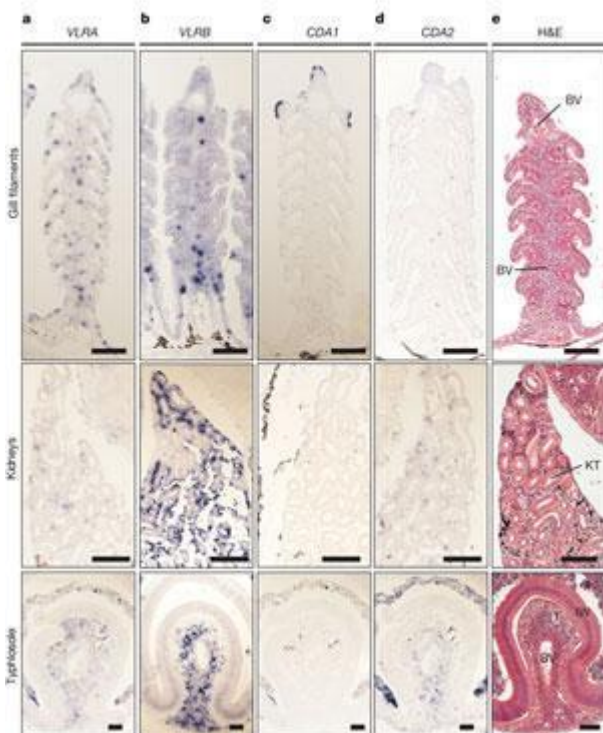
Anderson et al., showed that Aire regulates the expression of a large number of promiscuously expressed genes in mTEC.

Science 298:1395-1401, 2002

2011
2011
Thymoid

In 2011, Bajoghli et al., found a "thymus-like lympho-epithelial structures, termed thymoids, in the tips of the gill filaments and the neighbouring secondary lamellae (both within the gill basket) of lamprey larvae"