Concluding Remarks and Perspectives

I hope this book has served as an overview of the thymus gland, from fundamental aspects, historical background, its ontogeny, maturation of T cells and thymic crosstalk, control of transcriptional gene expression and gene networking to the implication of *Aire* gene mutations in autoimmunity to preclinical and clinical approaches that could restore thymic function following clinically induced damage. Interestingly, the major functions of the thymus are well established and effector T lymphocytes correspond to a cell type most studied in immunology. Nevertheless, the thymus as an organ is still neglected. However, two of the fundamental properties of the immune system occur within this organ, i.e. the generation of diversity of TCR receptors through V (D) J recombination and the self-non-self distinction. In fact, immunologists have made significant advances in understanding of these fundamental properties. Still, research of the organ itself, i.e. its ontogeny, developmental biology, its ageing and involution, effect of stress or infections and control of thymic gene expression, emerged only recently. Important perspectives involving multidisciplinary disciplines are opened from now. Understanding biology of autoimmunity is a challenge for twenty-first century and this emphasis places research on thymus in priority. We have better understand the control of promiscuous gene expression (PGE), since it is the basis of the diversity of autoantigens and the induction of immunological tolerance and prevention of autoimmune diseases. More than one hundred of *Aire* gene mutations were described and associated to clinical manifestations of APECED (APS-1) syndrome. However, nothing is still known about the possible effect of non-lethal mutations in the *Fezf2* gene, which also is a controller of PGE in the thymus, on aggressive autoimmunity in man. More recently, a new genome-editing technology called Crispr-Cas9 system has emerged. As with other strategies for the therapeutic use of Crispr-Cas9 as in cancer cases, this opened up perspectives for its use in an eventual thymus intervention of patients seeking to correct *Aire* mutations. In addition, interventions pursuing the regeneration of the aged thymus or even the thymus of patients who have had infections or autoimmune diseases or who have undergone chemotherapy or radiotherapy are currently being investigated. Therefore, the thymus gland offers us a vast possibility for studies

G. A. Passos (ed.), *Thymus Transcriptome and Cell Biology*,
https://doi.org/10.1007/978-3-030-12040-5

ranging from fundamental research up to its therapeutic intervention whose aspects represent challenges for the next years.

Ribeirão Preto, November 2018 Geraldo A. Passos

Index

A
Acetylcholine receptor, 179, 218
Addison's adrenal deficiency, 3
Addison's disease, 11, 193, 195, 196, 199
Adhesion/adhesion molecules, vii, 5, 6, 38, 118–125, 132, 133, 138, 151, 216, 220, 222, 225, 228
Adult thymus organ culture (ATOC), 219
Age-associated thymic atrophy, 274
Aggressive autoimmunity, 219, 227
α4β1, 118
α5β1 integrin, 118
αβ- *vs.* γδ-lineage choice, 86, 88, 89, 98–99
Antisense RNA, 218
Apoptosis, 20, 46, 47, 49, 96, 133, 137, 172, 173, 217, 228, 247, 250, 251, 257, 258, 260
ArrayExpress, 218
Autoantibodies, 47, 83, 151, 170, 171, 195–199, 203, 204, 219, 303, 305
Autoimmune Addison's disease (AAD), 195, 201, 202
Autoimmune diabetes, 12, 264
Autoimmune polyendocrine syndrome type 1 (APS-1), 192–199
Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy syndrome (APECED), viii, 11, 169, 171, 175, 193, 219, 222, 223, 228
Autoimmune regulator (AIRE), viii, 9, 11, 13, 14, 28, 39, 45, 47, 50–53, 83, 151, 154–156, 159, 169–183, 192–205, 217–226, 228, 236–244, 260, 298
Autoimmune thyroiditis, 12
Autoimmunity, 11–13, 15, 46, 47, 51, 53, 55, 100, 136, 151, 158, 177, 192, 201, 203–205, 219, 223, 226–228, 236, 248, 262, 264–266, 296, 298, 303, 304
Autologous hematopoietic stem cell transplantation (AHSCT), 296–305
Autophagy, 46, 47
Autoreactive thymocytes, 158, 159, 161, 217, 218, 222, 227, 258
Autotoxicity, 6, 14

B
B cells, vii, 7, 38, 72, 76, 77, 81, 82, 91, 92, 94, 95, 160, 161, 192, 195, 221, 241, 261, 265, 299
Bcl11b, 75–77, 96
β-selection, 36–37, 69, 78, 79, 88, 97, 99, 127, 128, 137, 247
β5t, 28, 40, 41, 43, 44, 49, 51, 217
Bipotent thymic epithelial progenitor, 20
Bone marrow (BM), 68, 72–74, 76, 94, 98, 125, 133–135, 150, 152, 153, 159, 247, 251, 260, 265, 274, 280, 282, 283, 296, 297, 299, 301
Bone morphogenetic protein (BMP), 23, 24, 26, 40, 250, 252
Branchial cleft, 20
Bromodomain-containing protein 4 (BRD4), 180, 181, 220, 221

C
CCL25, 40, 48, 70, 94, 120, 125–130, 133–135, 137
CCR7, 70, 82, 94, 119, 125–130, 132, 228
CCRL1, 39, 42, 43

G. A. Passos (ed.), *Thymus Transcriptome and Cell Biology*,
https://doi.org/10.1007/978-3-030-12040-5

CD3, 69, 78, 97, 227, 251
CD4, 12, 28, 38, 67, 120, 150, 174, 199, 216, 247, 256, 278, 296
CD8, 12, 38, 67, 120, 150, 174, 216, 247, 256, 277, 297
CD29, 118, 121
CD80, 28, 39, 52, 151, 157, 159, 160, 173, 217, 258
CD205, 39, 41–43, 217
Cell-adhesion proteins, 222
Cell migration, 8, 117–138, 247
Cellular differentiation, 227
Central tolerance, 10, 12, 36–38, 84, 128–130, 137, 138, 151, 205, 216, 217, 222, 228
Cervical thymus (CT), viii, 226
Chemokines, 8, 28, 39, 47, 48, 70, 82, 94, 118, 122–128, 130, 131, 133–138, 158, 159, 192, 221, 228, 261, 297
Chemotaxis, 122, 123, 134–135
Chromatin, 53, 78, 83, 96, 171, 172, 177, 179, 182, 183, 200, 220, 221, 224, 227, 242, 262, 263
Chromatin relaxation, 182, 220, 221
Chronic mucocutaneous candidiasis (CMC), 170, 193–195, 199
Claudin, 28
Cortex, 21, 28, 29, 36–37, 41, 48, 53, 67–69, 82, 92, 121, 124, 126–130, 134–136, 150–154, 216, 217, 247, 260, 275, 297
Cortical thymic epithelial cells (cTECs), 28, 29, 36–38, 40–49, 51, 92, 134–135, 151, 154, 216–218, 224, 250, 260, 275, 297, 298
Corticomedullary junction (CMJ), 41, 67, 69, 72, 74, 122, 124, 126–128, 134–135, 150, 160, 216, 274, 297
Crispr-Cas9, 222, 223
Crosstalk/cross-talk, vii, 44, 150–162, 174, 216, 283
CXCL12, 48, 120, 122, 123, 125–128, 130–136
Cyclin D, 28
Cytokeratin, 39, 239, 240
Cytokines, 38, 54, 79, 80, 89, 91, 93, 100, 118, 131, 197–199, 252, 257, 259, 276, 279–280, 283, 297, 301, 303
Cytoscape, 241
Cytotoxic lymphocytes, 258

D

Delta-like ligand 4 (Dll4), 28, 39, 40, 48, 72, 74, 92, 93, 95, 96, 278, 281
Dendritic cells (DCs), viii, 9, 10, 38, 45, 49, 51, 70, 73, 75, 84, 95, 100, 128, 129, 134–135, 137, 151, 158, 159, 161, 198, 217, 218, 222, 260, 261, 278
Dicer, 263
Differentiation, viii, 4, 23, 36, 69, 117, 150, 173, 224, 251, 256, 275, 296
Di George congenital syndrome, 4
DNA, vii, viii, 171, 172, 175, 181–183, 199, 200, 205, 219–221, 224, 239, 242, 243, 258, 274, 301, 302
DNA-activated protein kinase (DNA-PK), 182, 220
DNA-damage response, 181, 182, 221
Double-negative (DN), 36–37, 42, 48, 67, 68, 80, 81, 85, 99, 100, 118, 120, 122, 123, 126, 127, 130, 131, 134–135, 137, 150, 216, 251, 252, 260, 297, 302
Double-positive (DP), 12, 36–37, 49, 68, 69, 79–82, 85–88, 92, 94–98, 120, 122, 123, 126–130, 133–135, 137, 150, 151, 153, 155, 159, 216, 217, 251, 260, 278, 297, 302

E

E11.5, 20, 21, 25
E14.5, 21
Ectoderm, 39
Ectopic proteins, 218
Electrotransfection, 226
Endocrine systems, 3, 218
Endocrinology, vii, viii, 3–5
Endoderm, 20, 21, 23–26
Endothelial cells (ECs), 38, 124, 125, 131, 250, 276, 278, 282
Ephrins, 21, 120, 133
Epigenetics, 80, 161, 178, 205, 220, 221, 243, 262
Epithelial cellular adhesion molecule (EpCAM), 151, 217
Epithelial network, 19
E proteins, 75–79, 82, 87, 88, 90, 96
Exons, 171, 193, 199, 222, 223
Extracellular matrix (ECM), 8, 38, 118–123, 132, 134–135, 137, 138, 222, 260
Eya1, 23

F

Fetal thymus organ culture (FTOC), 26, 85, 91, 93–97, 219, 226, 251
FGF8, 24
Fibroblast growth factors (FGF), 23–26, 38
Fibronectin, 118–120, 122, 123, 125, 127, 132–137, 260

Forebrain-expressed zinc finger 2 (FEZF2), viii, 9, 12, 14, 39, 83, 84, 151, 175, 224
Forkhead-box N1 (Foxn1), 14, 20, 21, 23, 25–29, 40, 41, 48, 53, 250, 261, 275, 282, 298
Forkhead-box N4 (Foxn4), 14
Forkhead box P3 (Foxp3), 100, 150, 250, 256–259, 262–264, 303
Foxg1, 25
Fugetaxis, 123, 132, 134–135

G
Galen, vii, 2
γδ T-cells, 68, 86–92, 95, 98, 99, 101
GATA3, 72, 75, 76, 79, 81, 82, 96
Gene expression, viii, ix, 11, 50, 52, 77, 78, 83, 99, 100, 131, 160, 172, 175, 181, 182, 198, 201, 216–228, 237–238, 241–243, 248, 250–252, 262, 264, 275, 276
Gene expression networks, 236, 237, 241, 242
Gene Expression Omnibus (GEO), 218
Gene-gene expression relationship, 237, 240–243
Gene transcription, 11, 28, 183, 220, 276
Genome, vii, viii, 76, 151, 172, 178, 183, 201, 205, 217, 219, 223, 228, 263
Glial cells missing homolog 2 (Gcm2), 20, 25, 26, 40
Glutamic acid decarboxylase 65 (GAD65), 12, 14, 197
Glutamic acid decarboxylase 67 (GAD67), 13, 14
Graves' thyroid disease, 3

H
Hematopoietic stem cells (HSC), 70, 265, 281, 283, 296–305
High affinity, 38, 49, 82, 84, 137, 150, 158, 217, 257, 258, 260
History of the thymus, 2–15
H3K4me3, 183
HLA, 195
Homeostasis, viii, 11, 44–46, 48, 86, 160, 203, 227, 228, 250, 252, 261, 263, 266, 276, 300, 303
Horror autoxicus, 6
Hoxa3, 23
Hypoparathyroidism, 11, 170, 193–195, 197, 201
Hypoplasia of the thymus, 4

I
Icam4, 222
IL7, 25, 28
Immune-(neuro)endocrinology, 5, 218
Immune resetting, 299, 300, 302
Immunodeficiency, 4, 55, 203, 264, 265
Immunoperoxidase, 218
In situ hybridization histochemistry, 218
Insulin, 10–12, 14, 46, 50, 84, 155, 176, 197, 218, 304
Insulin-like growth factor (IGF), 38, 218
Interferon Regulatory Factors (IRF), 47, 48

J
Juvenile idiopathic arthritis (JIA), 304

K
Knockout mice (Aire-/- KO), Aire KO, 171, 175, 176, 219, 222

L
Lama 1, 222
Laminin, 118, 121, 122, 125, 127, 133–135, 138, 260
Long noncoding RNAs (lncRNAs), 220, 225–227, 263, 264
LTβR, 44, 45
Luteinising hormone, 54
Ly51, 151, 217
Lymphocytes, vii, 2–4, 7, 8, 10, 14, 15, 36, 40, 131, 132, 136, 152, 170, 171, 192, 227, 241, 247, 250, 255–266, 296
Lympho-stromal interactions, 153

M
Macrophages, viii, 38, 118, 134–135, 158, 161, 218, 250, 260, 261
Major histocompatibility complex (MHC), 7–10, 12, 13, 36, 39, 42, 48, 69, 79, 80, 82, 84, 86, 118, 138, 150, 151, 173, 216, 257, 259, 260, 275, 298, 301
Maturation, vii, viii, 21, 24–26, 28, 29, 36–39, 43, 46, 47, 51, 52, 67–101, 117, 118, 130, 131, 133–135, 138, 152, 159–161, 179, 217, 225, 252, 257, 261, 262, 264, 275
Medulla, 2, 10, 21, 28, 29, 36, 49, 50, 53, 68, 69, 82, 92, 100, 118, 121, 122, 124, 126–131, 134–137, 150–157, 159, 160, 174, 179, 216, 247, 260, 297

Medullary compartments, 150, 152, 154
Medullary thymic epithelial cell (mTEC), vii, 5, 28, 29, 36–38, 40–53, 82–84, 92, 118, 129, 134–135, 151–161, 173–176, 178, 216–224, 226, 228, 239, 250, 257, 260–262, 275, 297, 298
Mesenchyme, 20, 21, 23–26, 39, 276
Methylated H3K27, 83
Microarray, viii, 83, 218, 239, 243, 249
MicroRNA (miRNA), 54, 175, 176, 220, 221, 225, 226, 228, 236, 240–243, 263, 264
Migration, 7, 9, 20, 21, 25, 38, 40, 48, 92, 117–138, 150, 154, 225, 227, 247, 260, 265
Miller, J., vii, 3, 4, 36, 152, 278, 296, 302
miR-29a, 54
miRNA-mRNA, 226, 228
Module-trait association, 237
Monoclonal antibodies, 121, 218
Monocytes, 198
Morphogens, 22–26, 278
Mouse (*Mus musculus*), viii, 27, 29, 39, 40, 49, 50, 54, 67–101, 121, 122, 132, 136, 137, 151, 153, 155, 160, 171, 175–178, 216, 219, 223, 226–228, 275, 279
mRNA, 50, 54, 83, 176, 182, 201, 216, 218, 221, 222, 225, 226, 228, 239, 252
mRNA processing, 182, 221
mTOR, 47
Mucocutaneous candidiasis, 170, 193, 194
Mucosal-associated invariant T-cells (MAITs), 69, 82, 100, 101
Multiple sclerosis (MS), 47, 198, 205, 302, 303
Mutations, viii, 11, 14, 25, 45, 50, 73, 89, 90, 170, 172, 192–205, 222, 223, 228, 256, 264, 265

N
Naïve, 14, 81, 82, 89, 100, 274–279, 281, 296
Natural regulatory T-cells (nTreg), 150, 151, 155, 158, 160, 256, 296, 298, 299, 305
Negative selection (NS), vii, 7, 9, 10, 13, 36–37, 39, 50, 68, 69, 82–84, 92, 97, 128–131, 134–138, 150, 151, 158–161, 176, 177, 179, 192, 205, 217, 221–223, 226, 228, 247, 250, 257, 259–261, 296, 298, 301
Neural crest (NC), 20, 21, 23–25, 39
Neuroendocrine, 4–6, 9–11, 13, 15
Neuroimmunoendocrinology, 218
Neuroimmunomodulation, 5, 6
Neurophysin, 5
Neuropilins, 134
Non-coding RNA (ncRNA), 77, 175, 218, 225, 226
Nono-obese diabetic mice (NOD), 11, 46, 132, 136, 158, 176, 227, 264
Notch signaling, 71–76, 85–88, 91–96, 98, 99, 101
Nuclear factor κB (NF-κB), 45, 158, 258
Nuclear localization signals (NLS) domain, 172, 180, 222
Nuclear transport, 182, 221, 242

O
Online Mendelian inheritance in man (OMIM), 193, 219
Ontogeny, viii, 89, 299
Open-reading frame (ORF), 225, 226
Organogenesis, 26–29, 39–42, 45, 227, 275
Oxytocin, viii, 5, 6, 8–11

P
p53, 46, 283
Paired box 1 (Pax 1), 24, 25
Paired box 3 (Pax 3), 24, 25
Paired box 9 (Pax 9), 24
Pancreas-infiltrating T lymphocytes (PILs), 227, 228
Parathyroid, 20, 21, 24–26, 39, 40, 195, 196, 274
Pathogen-associated molecular patterns (PAMP), 54
Pavlovian reflex, 5
Pearson's correlation coefficient, 237, 241
Pericytes, 131
Peripheral tissue antigens (PTAs), 137, 204, 216, 217, 219–223, 225, 226, 298
Pertussis toxin (PTX), 124
Pharyngeal endoderm, 20, 24–26
Pharyngeal pouch, 20, 23, 24, 26, 39, 40
Pharynx, 20, 21, 24–26
Plant homeodomain (PHD1), 172, 178, 198–201, 220
Positive selection (PS), 36–39, 48, 49, 69, 77, 79–83, 85, 100, 129, 130, 150, 151, 154, 216, 251, 260, 298
Post-transcriptional control, 175, 221, 226
Post-transcriptional interaction networks, 226
Progenitors, 20, 41, 70, 124, 150, 174, 226, 250, 260, 280, 296
Promiscuous gene expression (PGE), viii, ix, 50, 53, 175, 177, 216–221, 223–225, 228
Psmb, 28

Psychoneuroimmunology, 5
pTEFb, 181, 220, 221
Puberty, 3, 54, 243, 274, 298

R
Receptor activator of nuclear factor kappa-B (RANK), 43–45, 48, 83, 156, 157, 174, 179
Recombination activating genes 1 (RAG1), 10, 14, 15, 69
Recombination activating genes 2 (RAG2), 10, 14, 15, 69, 251
Regulatory T cells (Tregs), 9–11, 13, 36–39, 46, 47, 49–51, 69, 84, 99, 100, 128, 132, 134–135, 150, 177, 192, 205, 217, 219, 247, 250, 255–266, 301–305
RNA, viii, 15, 77, 83, 120, 131, 175, 177, 180, 181, 198, 218, 220–222, 224, 225, 227, 228, 263
RNA-fluorescent *in situ* hybridization (RNA-FISH), 222
RNA Pol II, 220, 221, 224, 225
RNA-sequencing (RNA-seq), 151, 160, 216, 218, 227, 277
RORγt, 81, 82, 89
RT-qPCR, 178, 222, 236, 239, 243
Runx3, 79, 80, 82, 96, 99

S
Self-antigens (Self-Ags), 7, 9, 13, 36–37, 69, 82, 158–161, 169–183, 197, 217, 222, 261, 297, 298, 301, 305
Self-non-self, viii, 228
Self-peptides, vii, 8, 10, 12, 14, 15, 38, 48, 49, 79, 84, 150, 216, 220, 257
Self-representation, viii, 219–221
Self-tolerance, 2–15, 45, 46, 49–53, 100, 129, 266, 298
Semaphorins, 133–135
Sexual dimorphism, 236, 242, 243
Shh, 20, 23–25
Single-positive (SP), 36–38, 44, 47, 68, 69, 80, 85, 92, 95, 97, 99, 118, 122, 124, 126, 128–135, 137, 150–161, 216, 251, 259, 260, 297, 302
Sirt-1, 221
Six 1/4, 23
Small interfering RNA (siRNA), 222, 225, 226
Somatostatin, 50, 218
Stat3, 45, 131
Stochastic, 178, 217, 221
Stroma, vii, 36–39, 45, 53, 159, 227, 296
Super-enhancers, 178, 221, 263
Systemic lupus erythematosus (SLE), 47, 305
Systemic sclerosis (SSc), 303, 304

T
Tbet, 82
Tbx1, 23, 25, 27
T-cell factor 1 (TCF1), 76
T-cell repertoire (TCR), 49, 87, 117–138, 151, 159, 160, 236, 296, 300, 303, 305
TCR signaling, 78, 85–87, 89–91, 252, 261, 263
TGFβ, 23, 80, 100
Thoracic thymus (TT), 226
Thymectomy, vii, 3, 4, 152, 255
Thymic crosstalk, vii, 44, 150–162, 216
Thymic epithelial cells (TECs), vii, viii, 6, 8, 9, 12, 14, 21, 23–29, 36–55, 74, 92, 118, 120–123, 125, 128, 129, 134, 151, 180, 216, 217, 223–227, 236, 241, 243, 248–250, 252, 274–276, 278, 280–283, 297
Thymic medulla, 10, 21, 49, 50, 121, 124, 126, 127, 129, 247, 297
Thymic nurse cells (TNC), 5, 8, 121, 122, 127, 217
Thymic rejuvenation, 54, 296, 299–305
Thymocyte emigration, 130–132
Thymocytes, 3, 21, 36, 69, 117, 150, 173, 216, 241, 248, 257, 274, 296
Thymopoiesis, 9, 38, 40, 48, 55, 248, 250–252, 275, 276, 278, 280–283, 296–298
Thymoproteasomal processing, 220
Thymoproteosome, 28
Thymus, 2, 20, 36, 67, 117, 150, 170, 192, 216, 236, 247, 256, 274, 296
Thymus cunula, 2
Thymus-dependent (T) lymphocytes, 4
Thymus involution/thymic involution, 40, 53, 54, 205, 226, 274–277, 280, 282, 296, 298
Thymus-parathyroid primordium, 21, 40
Thymus-seeding progenitors (TSPs), 36–38, 48, 67–72, 74, 91–95
Tissue restricted antigens (TRAs), 10, 13, 46, 47, 50–53, 129, 151, 173–179, 183, 192, 204, 205, 236
Tissue specific antigen (TSA), 10, 83, 84, 236
T-lineage specification, 67–101
Tolerance, 7, 38, 84, 128, 150, 170, 192, 216, 236, 247, 261, 296
Tolerogenic, 10, 12, 14
Topoisomerase IIa (TOP2A), 182, 220, 221

Topological overlap matrix (TOM), 237
Transcriptional start sites (TSSs), 77, 178, 179, 220, 221
Transcription factors (TF), 12, 14, 20, 22–27, 40, 74–77, 79–83, 99, 101, 150, 151, 172, 179, 180, 220, 224, 225, 256–258, 262, 263, 275, 298
Transcriptome, viii, 46, 216, 218–219, 222, 228, 236, 247–252, 264, 275
Transcriptomic analysis, 160, 226
Trypanosoma cruzi (*T. cruzi*), 131, 136
Tumor necrosis factor (TNF), 156, 157, 174, 258, 259
Type 1 diabetes mellitus (DM-1), 227, 265

U
UEA1, 217, 218, 222

V
Vaccination against T1D, 12
Vcam1, 222
V (D) J recombination, vii, 217, 228, 260
Vestigial organ, 2–15
VLA-4, 118–120, 133
VLA-5, 118, 119, 125, 132, 133, 137

W
Wingless-related MMTV integration (Wnt), 23, 25–28, 40, 48, 276

Z
Zebrafish thymus, 26