

Joseph G. Sinkovics
CYTOLYTIC IMMUNE LYMPHOCYTES
in the Armamentarium
of the Human Host
Products of the Evolving Universal Immune System

www.schenkverlag.com
www.dialogcampus.hu

Joseph G. Sinkovics

CYTOLYTIC
IMMUNE LYMPHOCYTES

IN THE ARMAMENTARIUM OF THE HUMAN HOST
PRODUCTS OF THE EVOLVING UNIVERSAL IMMUNE SYSTEM

SCHENK VERLAG ❖ Passau
DIALÓG CAMPUS KIADÓ ❖ Budapest

Die Deutsche Bibliothek verzeichnet diese Publikation
in der Deutschen Nationalbibliographie;
detaillierte bibliographische Daten sind im Internet
über <http://dnb.ddb.de> abrufbar.

ISBN 978-3-939337-57-7

© Schenk Verlag GmbH, Passau, 2008
© Dialóg Campus Kiadó, Budapest, 2008

Editor: dr. Borbála Schenk • Layout: Zsolt Király

Das Werk einschließlich aller seiner Teile ist urheberrechtlich geschützt. Jede Verwertung außerhalb der engen Grenzen des Urheberrechtsgesetzes ist ohne Zustimmung des Verlags unzulässig und strafbar. Das gilt insbesondere für Vervielfältigungen, Übersetzungen, Mikroverfilmungen und die Einspeicherung und Verarbeitung in elektronischen Systemen.

All rights reserved.

Printed in Hungary

TABLE OF CONTENTS

TABLES	VIII
FIGURES (PLATES)	IX
KEY WORDS	X
PREFACE & INTRODUCTION	XI
FOREWORD (KATALIN PALOCZI)	XIII
PREFACE (H. DAVID KAY)	XV
A PREFACE FROM A CLOSE COLLABORATOR AND FRIEND (JOSEPH C. HORVATH)	XXII
1. THE INNATE IMMUNE SYSTEM	1
1.1. The Universal Ancestor(s)	1
1.2. Chemokines and Toll-like Receptors Make Their Appearance	7
1.3. Nod	12
1.4. Viral Evolution	13
1.5. Interferons and Small Interfering RNAs	17
1.6. The Ascidian Haemocyte and Its Followers	24
1.7. Programmed Cell Death	25
1.8. Bactericidal Substances	39
1.9. Some Simple Interactions	39
1.10. Oncogenic Mutations	40
2. THE ADAPTIVE IMMUNE SYSTEM	43
2.1. Primordial Adaptive Elements	43
2.2. Retrotransposons	44
2.3. Host and Viral Genetic Refinements	45
2.4. The Placenta	63
2.5. The Thymus, Bursa of Fabricius and MALT	67
2.6. The Host Confronts Mutagens and Pathogenes Attacking Lymphoid Tissues	69
2.7. DC, NK, T, B, T _{REG} Cells	73
2.8. Humoral and Cell-Mediated Immunity	75

3. TUMOR IMMUNOLOGY AND IMMUNOTHERAPY	89
3.1. Cancer Vaccines	89
3.2. Natural and Artificial Hybridoma Formations by Cell Fusions	92
3.3. Lymphocytes Cytotoxic to Human Tumor Cells	115
3.4. Adoptive Immune Lymphocyte Therapy	128
4. AUTOIMMUNITY AND LYMPHOMAGENESIS	137
4.1. Hashimoto Thyroiditis	137
4.2. Systemic Lupus Erythematosus	140
4.3. Malignant Thymoma and Autoimmunity	144
5. CYTOTOXIC LYMPHOCYTES AGAINST CELLULAR AND VIRAL INFECTIOUS AGENTS	149
5.1. Complex Eukaryotic Pathogens	149
5.1.1. <i>Worms</i>	149
5.1.2. <i>Unicellular Parasites</i>	151
5.1.3. <i>Fungal Pathogens</i>	156
5.2. Septic Shock	157
5.3. Interaction with Intracellular Bacteria and Mycobacteria	159
5.3.1. <i>Bacteria</i>	159
5.3.2. <i>Mycobacteria</i>	163
5.4. Anti-Oncoviral Immunity	170
5.4.1. <i>Retroviruses</i>	170
5.4.2. <i>DNA Viruses</i>	187
6. A CONCISE SYNOPSIS (2007-8)	215
6.1. From RNA Concentrates to Synthrophus and Upward	215
6.1.1. <i>Another Ancient Microorganism</i>	215
6.1.2. <i>The RNA Prevails</i>	216
6.1.3. <i>Avian Retroviruses and Herpesviruses Compete for the Malignant Transformation of Their Host Cells</i>	218
6.1.4. <i>Unicellular Eukaryotes Emerge and Evolve</i>	219
6.1.5. <i>Transposable Elements Sustain the Plasticity of the Genomes</i>	221
6.1.6. <i>The Pluripotent Stem Cell</i>	224
6.2. Innate Immune Faculties Regulate the Lymphocytes	225
6.2.1. <i>Land Plants Derive from Green Algae and Develop Defensive Reactions</i>	225
6.2.2. <i>Circulating Cells Arise in the Service of Multicellular Hosts</i>	226
6.2.3. <i>Molecular Defensins Predating Antibodies</i>	227
6.2.4. <i>Subverted Cytokines of the Host Serve the Tumor</i>	230
6.2.5. <i>The Most Advanced Hosts Still Mobilize Innate Defenses</i>	231

6.3. Innate and Adaptive Interactions in Infections and in Malignancies	233
6.3.1. <i>CpG Islands</i>	233
6.3.2. <i>The Pathogens Strike Back. Treacherous Cytokines</i>	234
6.3.3. <i>The Immunosuppression of Pregnancy</i>	236
6.3.4. <i>Co-evolving Viruses Establish a “Criminal Collusion”</i>	241
6.3.5. <i>The Immuno-evasive Skills of the Viral Genome is Matched by Protozoa</i>	256
6.4. Latest Events in Human Cancer Immunology and Immunotherapy	257
6.4.1. <i>Epigenetics</i>	257
6.4.2. <i>Ancient TLRs Knew How to Induce Apoptosis</i>	258
6.4.3. <i>Obstacles to Antitumor Immune Reactions of the Host</i>	259
6.4.4. <i>Cancer Vaccines Induce Antitumor Immune Reactions but the Tumor Survives</i>	261
6.4.5. <i>Adoptive Immune Lymphocyte Therapy</i>	262
SUMMARY	269
APPENDIX I.	281
APPENDIX II. (ADDED AT PROOF-READING)	285
AUTHOR’S POSTSCRIPT	293
REFERENCES	295
ACKNOWLEDGEMENTS	377
ABOUT THE AUTHOR	381
SUBJECT INDEX	383

TABLES

Table 1. <i>Viral Evolution</i>	15
Table 2. <i>Subversion of Chemokines and Cytokines by Malignant Cells. Some Agents and Interventions Counteract</i>	21
Table 3. <i>Involvement of Interferons in Innate and Adaptive Anti-Tumor Immunity</i>	23
Table 4. <i>The FasL → FasR Paradox</i>	26
Table 5. <i>Human Endogenous Retroviruses and Immune Reactions</i>	62
Table 6. <i>Human NK Cells</i>	76
Table 7. <i>Graft-versus-Host Disease Reduced Death Rates and Prolonged the Course of Viral Mouse Leukemia</i>	131
Table 8. <i>Late Survivors with or without Leukemia in Leukemic Mice which Received Irradiation and Hemato-Lymphopoietic Cells from Leukemia Virus-Immunized Donors</i>	132
Table 9. <i>Viral Leukemogenesis in Mice Rendered Tolerant to, and Treated with, Allogeneic Lymphoid Cells</i>	133
Table 10. <i>Anti-Cancer Adoptive Immune Lymphocyte Therapy</i>	134
Table 11. <i>Suppressor Lymphocytes Counteracting Immune T Cells</i>	135
Table 12. <i>Synopsis of Oncoviral Phylogeny & Immunology</i>	274
Table 13. <i>Host Innate Immune Faculties React to Tumors. Tumors Subvert Host Immune Faculties</i>	277
Table 14. <i>Recent Development in Human Cancer Immunotherapy</i>	279
Table 15. <i>A Brief Summary of the Viral Therapy of Human Cancers</i>	281
Table 16. <i>Author's Experience with Viral Oncolytic Cancer Vaccines and with Immune Lymphocyte Therapy</i>	282

FIGURES (PLATES)

Figure 1. <i>Ribozymes Interact in Vesicles with Self-Replicating Peptides and Oligonucleotides. Protocells. The Root of the Tree of Life</i>	2
Figure 2. <i>Within the First Protocell</i>	3
Figures (Plates) 3. <i>Programmed Cell Death and Apoptosis</i>	27
Figures (Plates) 4. <i>Filamentous Cytoplasmic Structures Mistaken for Protoviruses. Endogenous Retroviruses Bud. The Looks of HIV-1</i>	47
Figures (Plates) 5. <i>Small Compact (Immune T) Lymphocytes. Large Granular (Natural Killer Cell) Lymphocytes</i>	77
Figures (Graphs) 6. <i>Growth Curves of Tumor Cells in the Chamber-Slide Assay Respond to Exposure to Lymphocytes and Antibodies</i>	81
Figures (Plates) 7. <i>The First Specific-Antibody-Secreting Natural Hybridoma</i>	97
Figures (Plates) 8. <i>Human Lymphoma Cell Line Imitates Natural Hybridoma Formation. Sézary Cells of Mycosis Fungoides</i>	101
Figure (Plate) 9. <i>VSV Is an Oncolytic Virus</i>	114
Figures (Plates) 10. <i>Fibroblasts Protect and Feed Lymphocytes. Lymphocytes Attack and Kill Malignantly Transformed Cells</i>	117
Figures (Plates) 11. <i>Herpesviruses (EBV/HHV-4; KSHV/HHV-8) Induce Malignant Transformations</i>	194
Figures (Plates) 12. <i>Cell-Free Fluids from Human Sarcoma Cell Cultures Induce Focus Formation and Antigenic Conversion in Human Embryonic Fibroblast Monolayers but Do Not Yield Retroviral Isolates. The Cytoplasmic Tubuloreticular Structures</i>	242

KEY WORDS

origin of living matter ♦ origin of cells ♦ origin of viruses ♦ innate immune systems ♦ Toll-like receptors ♦ chemokines ♦ cytokines ♦ bactericidal substances ♦ defensins, pentraxins, human neutrophil peptides ♦ interferons ♦ interleukins ♦ RNA interference ♦ retrotransposons ♦ programmed cell death ♦ apoptosis ♦ Fas ligand & receptor ♦ death domains ♦ origin of genes encoding adaptive immune faculties: V(D)J RAG RSS ♦ adaptive immunity evolving ♦ bursa ♦ thymus ♦ dendritic cells ♦ NK cells ♦ regulatory T cells ♦ cytotoxic T lymphocytes ♦ myeloid-derived suppressor cells ♦ fused tumor cells ♦ natural hybridomas ♦ monoclonal antibodies ♦ placenta immunology ♦ oncogenes and oncoproteins ♦ gene methylation ♦ histone de-acetylation ♦ CpG islands ♦ pluripotential cells ♦ endogenous human retroviruses ♦ autoimmunity in lymphomagenesis ♦ HIV-1 immunology ♦ antiviral immunity ♦ immune reactions to pathogens ♦ anti-bacterial/fungal immunity ♦ septic shock ♦ oncogenic viruses ♦ “criminal collusion” of oncogenic retro- and herpesviruses ♦ anti-oncoviral (HTLV, HPV, EBV, HHV-8) immunity ♦ Kaposi’s sarcoma ♦ Reed-Sternberg cells ♦ innate and adaptive immune reactions to cancer ♦ cancer immunotherapy ♦ oncolytic viruses ♦ viral therapy of human cancers ♦ viral oncolysates cancer vaccines ♦ cytolytic T lymphocytes ♦ immuno-evasion ♦ adoptive immune lymphocyte therapy ♦ overcoming obstacles of immunotherapy

1. THE INNATE IMMUNE SYSTEM

1.1. The Universal Ancestor(s)

In the prebiotic era, over three billion years ago, chemical reactions produced **nucleotides and amino acids** underwater in the hydrothermal vents (**6.1.1**), or on the surface of the ancient Earth. Some of these elements might have arrived to the Earth *via* meteorites. The “RNA World” established itself [1, 2]. Coacervates of Oparin [3, 4], or chemotons of Gánti [5, 6], or so called “self-perpetuating structural states” [7] accepted the entry of the ribozymes of Cech [8], which performed the Eigen and Ghadiri reactions [9, 10] with preformed RNA nucleotides and amino acids within these microscopic vesicles [11] (the author’s proposition in **Figures 1, 2**). Hammerhead ribozymes might have functioned similarly on their own in the microenvironment of clay-mineral structures [12, 13]. Living matter might have been generated more than once, but only its RNA/DNA-dependent form survived on the Earth. Descendants of the ancient hair pin and hammerhead ribozymes exist in some plant viruses, viroids and their satellites (cited in reference [11]). The universal ancestors of all later life forms on the planet Earth have come to existence, lived in conglomerates, resembling those of extant *Mycoplasmataceae*, or colonies of L-forms or spheroplasts, competed for, and exchanged, their primitive genes horizontally, and inherited them vertically [11, 14-16]. Archaea, eubacteria/prokaryotes and their viruses populated the ancient anaerobic and overheated Earth [11, 14-17].

Bacteria and later eukaryotes formed their **cell membranes** from ester-linked fatty acids, while their predecessors, the *Crenarchaea* (*Sulfolobales*) and *Euryarchaea* (*Haloarchaea*), composed their cell membranes from ether-linked isoprenoid core lipid bilayers [18, 19]. Before, and even after cell wall formation was established, cell fusions occurred and resulted in the formation of **nucleated eukaryotes** from events of eubacterial-archaeobacterial symbioses (**Figure 1**), when the eubacterial partner possessed membrane-bound fibrillar double-stranded dsDNA-containing nucleoids [15]. One survivor of such eubacteria is the *Gemmata obscuriglobus* [20].

According to the author’s proposition, **ancient fusogenic viruses** (like phage L3 of *Acheloplasma laidlawi*; *Fuselloviridae* of *Crenarchaeota*; ancestors of extant phages of *Mycoplasmataceae*) might have mediated the fusion of prokaryotic and archaeal spheroplasts, giving rise to the first eukaryotes [11, 16]. Viruses enabled to fuse cells remained active up to this date (**3.2**). Seminal events, such as these fusions, could be reconstructed today by subjecting extant

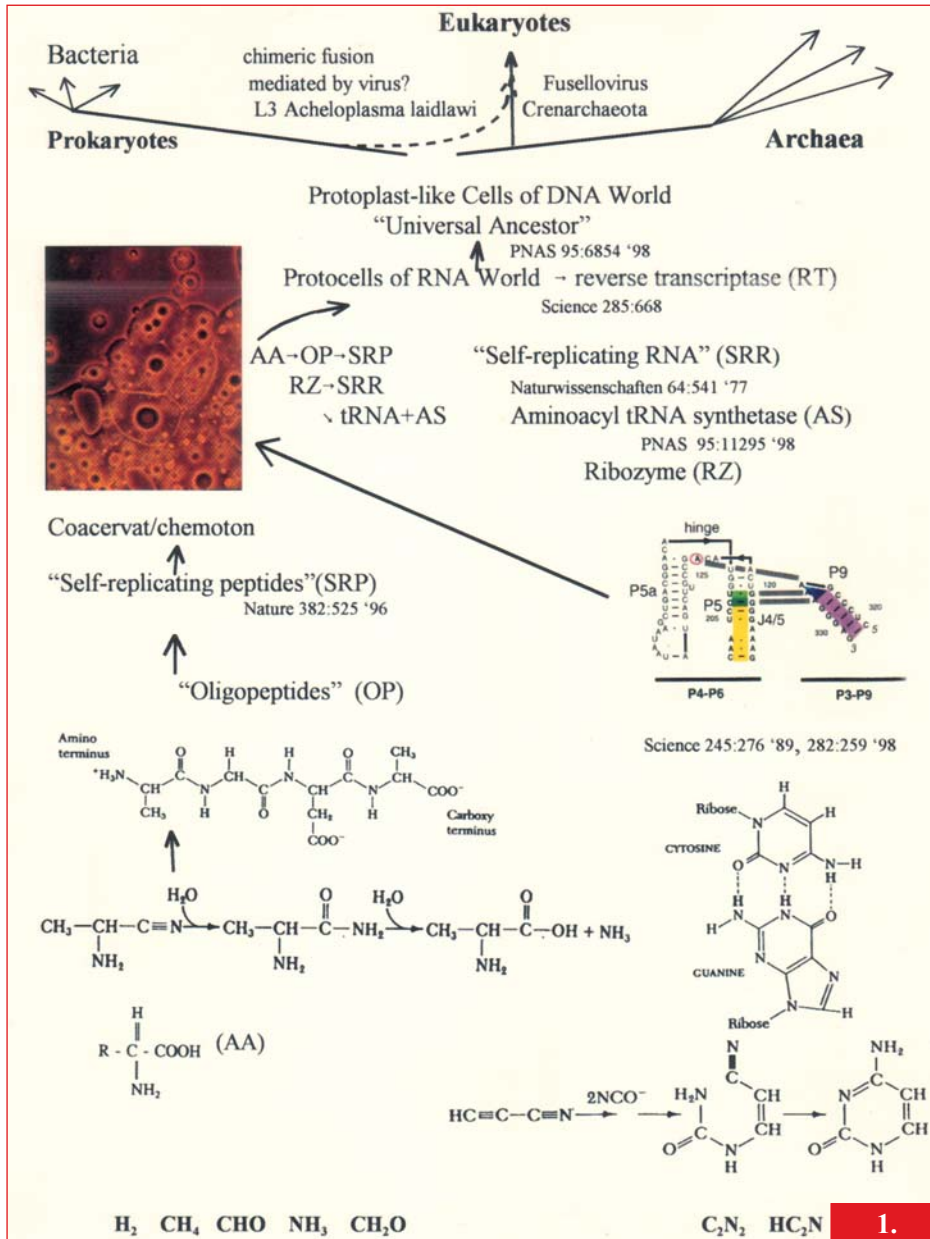
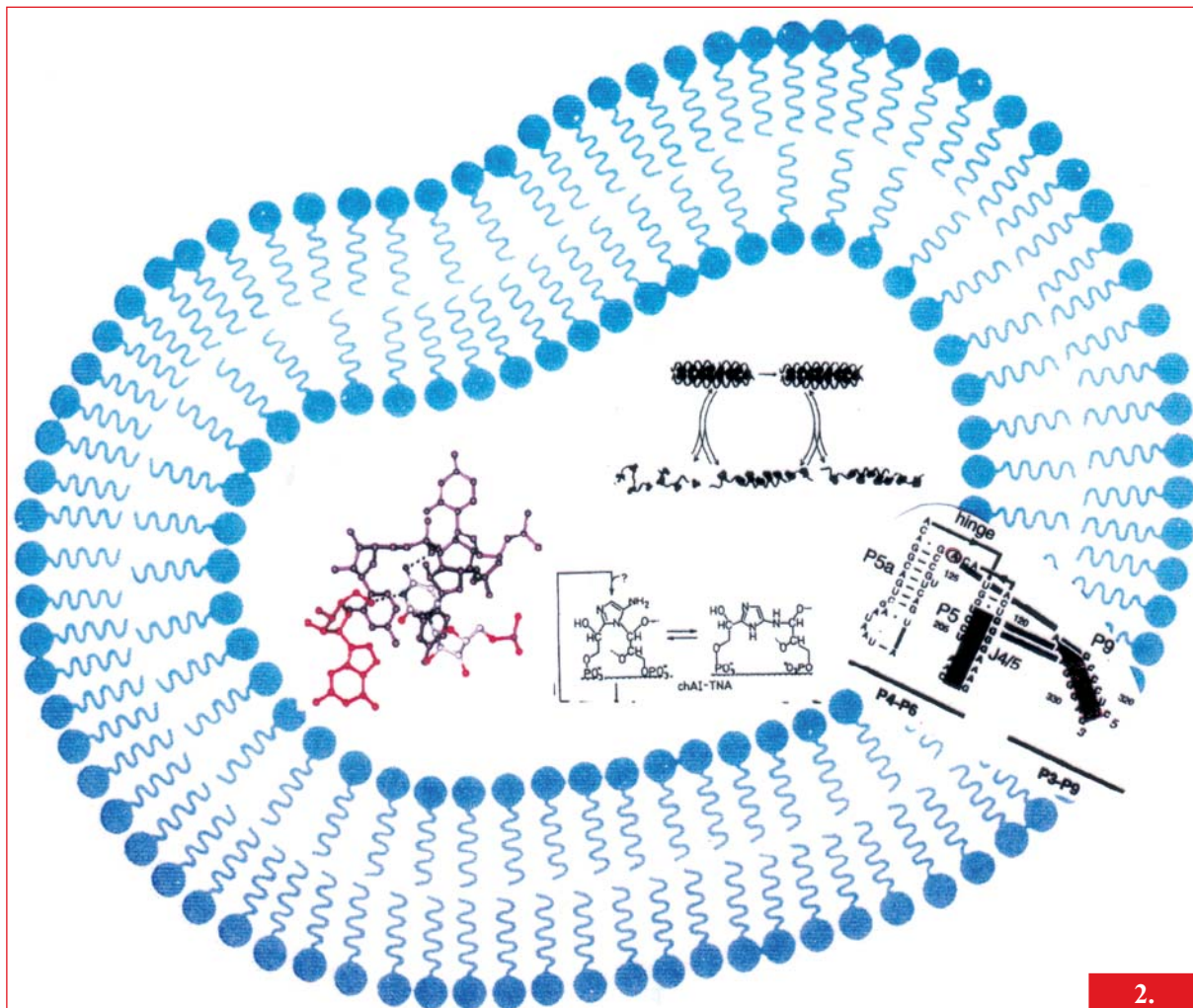


Figure 1. Lower left column from bottom up: prebiotic synthesis of amino acids to oligopeptides. Autocatalytic oligopeptides [10]. Vesicles with lipid membranes (like those deriving from the contents of the Murchison meteorite). Lower right column from bottom up: pre-biotic cytosine nucleotide. Ribose; the RNA World [1, 2]. Ribozyme [8]. Long oblique arrow: ribozyme aiming at lipid-membraned vesicle for entry. Upper segment: self-replicating RNA. Protocells of the RNA World. Reverse transcriptase: RNA → DNA. Protoplasts of the emerging DNA World divide into archaea and prokaryota (bacteria). Fusions of protoplast-like archaeal and prokaryotic cells result in nucleated cells (eukaryota). Fusogenic ancient virus mediates the fusion of archaeal and eubacterial protoplasts into eukaryotic cell [11, 16]. Organelle-free (no chloroplasts; no mitochondria) protozoa (*Giardia*-like) emerge (or if they had organelles, they lost them). Cells acquire chloroplasts from cyanobacteria (plant cells) and mitochondria from α -proteobacteria (unicellular protozoa) [15]



2.

Figure 2. Ribozyme (hair pin, hammerhead, ribonuclease P) enters membrane-bound vesicle (coacervate of Oparin; chemoton of Gánti; vesicle encased in amphiphilic bilayer of Bell) containing pre-formed oligonucleotides ready to replicate (the Eigen reaction) [9], and amino acids to form self-replicating oligopeptides (the Ghadiri reaction) [10]. Short replicating RNA sequences perform simple functions; independently evolved RNA population may coexist [1, 2, 12, 13]. This is the site where the primaevial RNA codes (RNY; R = purines, Y = pyrimidines, N = any of them) advanced into the extended RNA codes (RNY, NYR, YNR) in which each reading frames represent 16 triplets, altogether 48 triplets, specifying 17 of the 20 amino acids including the translation initiation codon AUG [2]. Thus, the general encoding function, mapping each codon to its corresponding amino acids, or to stop signal, is already functional. The universal ancestor (the last common ancestor of archaea, prokarya and eukarya) possessed reverse transcriptase and its standard genetic code evolved from the primaevial extended RNA code [14].

(At the time of their publication in *Studia Physiologica* 9:5-151, 2001, Professor Sándor Juhász-Nagy gave permission for any eventual reprinting of these figures in a future English language publication. The author is especially grateful to Professors Sándor Juhász-Nagy and Sándor Koch for their invitation to prepare and publish this article [11]).

archaeal and prokaryotic spheroplasts to fusogenic phages [11, 16]. *Mycoplasma* preserved the L3 phage, which induces large cell-conglomerates of these ancient microorganisms. L3 is a tailed phage with 40kb dsDNA genome; it exists in the form of several mutated entities, which recombine with each other. In the Max Planck Institute in Martinsried, Germany, *Mycoplasma* are placed to the bottom of the Tree of Life, below the primitive prokaryote *Aquifex aeolicus*. *Aquifex pyrophilus* gains electrons from hydrogen, carbon and thiosulfate at 86-90 °C and operates the ancestor of RNA polymerases (cited in [11]).

Before their courses separated, the archaea, prokaryotes, and the nucleated eukaryotic common ancestor(s) resisted radiation, acid and heat, practiced anaerobic Embden-Meyerhof glycolysis, possessed **histone-DNA genomes**, horizontally exchanged primordial genes, and synthesized proteins through mRNA and aminoacyl-tRNA synthetase obeying the universal genetic code [21,22]. Resembling those ancient cells, the cell membrane of the extant *Ferroplasma acidarmanus* preserves its integrity at pH 1 of sulfuric acid. *Deinococcus radiodurans* survives heavy gamma rays irradiation by rapidly repairing DNA damage. The archaea, *Pyrococcus furiosus*, remains alive and functional close to the boiling temperature of water. The primitive prokaryotes (*Aquifex*; *Thermotoga*) acquired 15-24% of their genes from archaea (cited in [11]).

Unicellular algae (*Chlorella*) acquired proton-activated O₂ producer **chloroplasts** from cyanobacteria. From these algae, plant cells evolved in the sea and continue to respond to photons after populating dry land. Extant chloroplasts still operate with RNA polymerases of bacterial, or T bacteriophage origin (cited in [11]). The engulfment of α -proteobacterial symbionts to form mitochondria in some unicellular eukarya (algae included) occurred, and animal cells possessing mitochondria, but not chloroplasts, evolved. **Mitochondria** promoted the switch to aerobic (Krebs) glycolysis. Some protists (*Entamoeba*; *Giardia*) do not seem to have mitochondria; however, they possess mitochondrial genes in their nuclei. These metamonada possessed mitochondria, but after expropriating the useful mitochondrial genes, they have gotten rid of them (but tiny relics of former mitochondria persist in their cytoplasm) (*vide infra*). The aerobic microbial ecosystem emerged [23], and co-existed with the ancient anaerobic life forms, which extended their life span up to the present. However, the purple aerobic anoxygenic phototrops of the sea, such as *Roseobacter denitrificans*, capture photons in the presence of O₂, and fix CO₂, but can not produce oxygen due to the loss from their mitochondria of α -proteobacterial derivation, the genes to encode the necessary enzymes of the Calvin cycle (ribulose biphosphate carboxylase and phosphoribulokinase) [24].

While mitochondria became oxygen-consuming and ATP-producing organelles, the mitochondria of **anaerobic eukaryotes** (in *Euglena gracilis*; in platyhelminths, nematodes, snails and mussels) produce ATP by proton-pumping electron transport without O₂ consumption. Derivatives of anaerobic mitochondria are the H₂-producer hydrogenosomes of ciliates, amoeboflagellates and parabasalids. The mitochondrial exoribonuclease-polymerase enzyme,

polynucleotide phosphorylase (PNPase), derives from ancient prokaryotes [25]. In plant cells, the enzyme functions in the chloroplast stroma. In higher eukaryotes (in mammalian cells), the enzyme promotes differentiation and senescence; it functions as a pro-apoptotic agent being an antagonist of the c-Myc and Bcl_{XL} anti-apoptotic proteins (1.7). Mitochondrial DNAs (mtDNA) have been sequenced, including those of human mitochondria. The mitochondria of protists (amoebae, flagellates, algae) and plant cells remain large and encode close to 100 proteins, whereas in the cells of metazoa, the mitochondrial genome is compacted into a small size, operating only a few genes, which encode less than 20 proteins for electron transport and/or oxidative phosphorylation. Great numbers of original mitochondrial genes were transferred into the nucleus of the metazoan host cells. Amoebozoa not yet, but Euglenozoa and Archazoa (parabasalia; metamonada) do already possess the dihydrofolate reductase and thymidylate synthase fusion enzyme (DHFR/TS), whereas ancestors of animalia and fungi (opisthokonta) are devoid of the fusion enzyme; instead, they received the elongation factor-1 α (EF-1 α) insertion. Amoebozoa separated from the tree of life before the acquisition of both DHFR/TS and EF-1 α [26-29]. Choanoflagellate protists and the unicellular eukaryotic ichthyosporeameosomycetozoa (opisthokonta) are the ancestors of the first metazoan colonies evolving toward animals and fungi, respectively [29-31]. While *Crenarchaeota* and *Euryarchaeota* accommodated a one millimeter long DNA strand (with the help of histones, as in *Methanothermus fervidus* [32]), advanced eukaryotes will have to compact one, two meters long, DNA strands in their nuclei.

The CO₂-consuming, **photosynthetic chloroplasts** of higher plants derive from the prokaryotic cyanobacteria: the chloroplasts are remnants of, reduced from free-living to endosymbiotic, cyanobacterial progenitors. Chlorophyll fluorescence (red fluorescence in response to UV light) is an indicator of photosynthetic performance. Chloroplast-related mutations affect leaf coloration. The free-living ancient prokaryote, cyanobacterium, is estimated to have operated well over 3000 genes, whereas chloroplasts of extant plants possess only circa 120 genes. Large numbers of chloroplast genes migrated into the nucleus of their host cells. In the genome of *Arabidopsis*, 18% of the genes are identified as of chloroplast derivation. Unicellular eukaryotes once in the distant evolutionary past possessed chloroplasts. In the extant descendants of these ancient eukaryotes, which have become parasites of metazoan hosts, identifiable chloroplast genes remain operational. Trypanosomatids (the causative agents of leishmaniasis, sleeping sickness, Chagas' disease) (5.1) operate enzymes, proteins and organelles that are encoded by genes of chloroplast derivation: peroxisome-targeting signals, aldolases, and glycosomes. An adenylylate kinase of *T. brucei* is almost identical with the enzyme operating in the chloroplasts of the maize; the fatty acid desaturase of the trypanosoma and that of the soybean are closely related. *Euglena gracilis* must have engulfed a green alga that already had a cyanobacterial endosymbiont. A fatty acid desaturase of *E. gracilis* is almost identical with the same enzyme operational in plants and fungi. The enigmatic "hohlzylinders" in the cytoplasm of plasmodia and toxoplasma,

are of cyanobacterial chloroplast derivation. Proton pump enzymes of toxoplasma and plasmodia are identical with those operating in the chlorophyte, *Chlamydomonas reinhardtii* [29, 33-37] (6.2). This chlamydomonas possesses the ancestor of the LI819 polypeptide-encoding gene, which is preserved in the photosynthetic eukaryotes, predating their division into green (chlorophyte), red (rhodophyte) and brown (chromophyte) algae [38]. The *cox3* gene encodes the subunit III of the enzyme cytochrome c oxidase. *C. reinhardtii* transferred this gene from its mitochondria into its nucleus. The colorless alga polytomella also carries this gene in its nucleus; therefore, polytomella separated from chlamydomonas after the the event of this gene transfer [39]. The mitochondrial *coxI* gene encoding subunit I of the enzyme cytochrome oxidase in another colorless alga, *Prototheca wickerhamii*, is structurally related rather to that of ascomycetous fungi and higher plants, than to that of *C. reinhardtii* [40], suggesting that these introns derived from ancient α -proteobacteria, the ancestors of all mitochondria.

Phagocytosis and inhibitory micro-RNAs [41] and the related **small interfering RNAs (1.5; 6.1.1)** protected the ancient unicellular organisms against bacteria and viruses, respectively, and remain active in all extant eukarya, especially in plants [42]. Ancient eukaryal cell colonies coalesced into organized multicellular organisms, setting the stage for the “cambrian explosion”. Intron insertions, splicing and spread (vertically and horizontally) formatted the ancestral genomes, far pre-dating those of the eukaryotes. There are self-splicing group I and group II introns, tRNA (archaeal) introns, and pre-mRNA nuclear spliceosomal introns. Introns interrupt exons and promote exon recombinations leading to the synthesis of new proteins. Group II introns extend their existence from archaea and bacteria through algae, fungi and plants. The tRNA introns of archaeal tRNA, rRNA, mRNA derivation are already in the nuclei of eukaryotic cells [43, 44]. Operons were formed by gene re-arrangements within the genome, or by acquisition of horizontally transferred genes from another species [45]. The ubiquitin-mediated signaling cascade of eukaryotes dates back to prokaryotes, and to the tail assembly gene cluster of their caudate phages [46]. mRNA translation starts from the AUG initiator codon from a viral replication in a host cell through photosynthetic cyanobacteria to the production of human proteins by *E. coli* [47, 48]; systems exist for the insertion of a stop codon into the mRNA not be translated, and external interventions (the antibiotic pactamycin) may arrest translation (i = interfering; m = messenger; r = ribosomal; t = transfer RNAs).

This most remarkable period in the evolutionary history of life forms on Earth withstood, and turned into advantages, the alterations of the **“immutable” universal genetic code** (example: the *leucine codon CUG* is decoded as serine SertRNA CAG in *Candida*) [49] (6.2) and the insertions of prokaryotic genomic sequences into metazoans. Examples are the appearance of the bacterial glyoxylate cycle malate synthetase and isocitrate lysase genes into the common ancestor of nematodes [50]; the recombination activation gene (RAG) sequences into sea urchins and *Placoderms* (2.2; 2.7). Genes exchanged be-

tween the ancestors of metazoa remain preserved and their protein products are recognized as “self” by the united innate and adaptive immune system. Not only malignantly transformed cells of the host, but exogenous pathogens can present proteins that escape immune recognition by the host, as if they were “self.” Ancient horizontal gene transfers and the recent evolution of the placenta compromised further the united innate and adaptive immune system (2.4). The two later events matching, or surpassing the achievements of the Cambrian explosion, are those of the development of the placenta forcing the adaptive immune system to accept the semi-allogeneic fetus, and compensate for the compromise; and the evolution of the human cerebral cortex, establishing the place, and determining the fate, of the human race in the Universe.

1.2. Chemokines and Toll-like Receptors Make Their Appearance

New life forms radiating out during the Cambrian explosion some 700-500 million years ago protected themselves by phagocytosis resulting in either the digestion, or in the walling off, the potential bacterial pathogens. This was the time, when the armada of **chemokines** and their G-protein-coupled receptors, arranging cytoskeletal re-arrangements, appeared [51-54] to recognize viral invaders and to direct cell migrations. Famous chemokines (IL-8, CXCL8; RANTES, CCL5; IP-10, CXCL10) (*vide infra*) emerge to enter into a close relationship with lectin-, lipopolysaccharide-, peptidoglycan-, flagellin-, mannan-, and nucleotide-recognizing **Toll-like receptors** (TLRs) [55-60]. “Toll!” in German (Deutsche Forschungsgemeinschaft): “I am delighted!” ”dieses Lied ist so toll;” “einzeln sind wir all toll;” “findet ihr die Texte so toll, so geil, so vielsagend;” “Brigit ist einfach toll;” “aber mit Deinen Sexleben, ist das aber nicht mehr toll?” “Toll” in American: “Pay transit fee, or else!” Generating business with the invitation of “toll-free calls.”

The ancient pathogens could not make Toll-free calls! The complex system of TLRs, capturing their ligands and reacting to “pathogen-associated molecular patterns” (PAMP) made its appearance in the first multicellular organisms. TLRs recognized pathogens, those attached to the cell surface, or those penetrating the cytoplasm (in the compartments of lysosomes and endosomes). Genes discovered much later, being functional in higher level eukaryotes (melanoma differentiation-associated gene-5, MDA5; retinoic acid-inducible cytoplasmic helicases, RIG-I), originated in the innate system and functioned as the principal agents for the recognition of RNA viruses [60]. The primordial system must have involved the **mediators of inflammation**: heat shock proteins, histamine, bradykinine and arachidonic acid metabolites (cyclooxygenases, prostaglandins, and leukotriens) [61-64]. The ancient system remains well conserved up to the highest ranks of vertebrates and mammals, where chemokines direct the movements of suppressor lymphocytes, TLRs immediately recognize Gram-negative, and Gram-positive bacteria and viral nucleic acid signatures; and

Table 4. The FasL→FasR Paradox

Fas ligand and receptor; Fas-associated death domain (CD95L; CD95; Apo-1; FADD/DISC) parts of the TNF family. N terminus of FADD recruits caspase 8 to proteolytically activate caspases-3/9 and induce apoptosis (**A**).

TNF →TNFR-1 activates anti-**A** nuclear factor NFκB, inducing pro-inflammatory cytokines. Transplants and tumor cells express FasL to kill FasR⁺ host lymphocytes. FasL⁺ tumor cells elicit a leukocyte- and antibody-mediated immune reaction. Via C' or ADCC reaction, the antibody kills FasL⁺ tumor (mouse melanoma) cells. One type of antibody-stimulates CD95Fas to activate not FADD, but mitotic pathway. Another type of agonistic antibody stimulates CD95Fas to induce **A**. ZB4 is a CD95 antagonist antibody [328-332].

Glioblastoma and melanoma cells operate *auto- or paracrine FasL → FasR circuits* for cell proliferation [110, 253-255].

In theory, in melanoma cells chromosomes broken at 10q23-26 (Fas locus) and at 1p32-34 (G-CSF locus) reunite attaching intracellular FasR domain to G-CSF pathway in t(1;10)(p32-34;q23-26) and resulting in mitosis at stimulation of FasR extracellular domain [254] (**1.5; 6.3.2**).

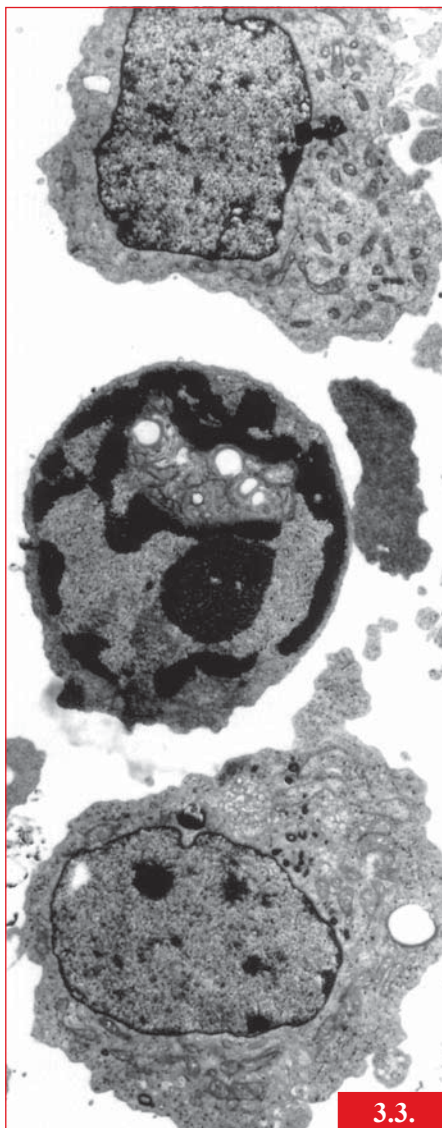
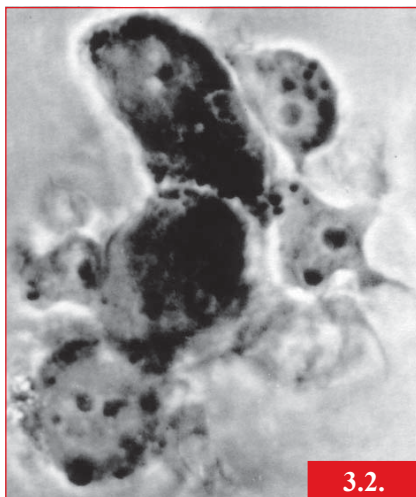
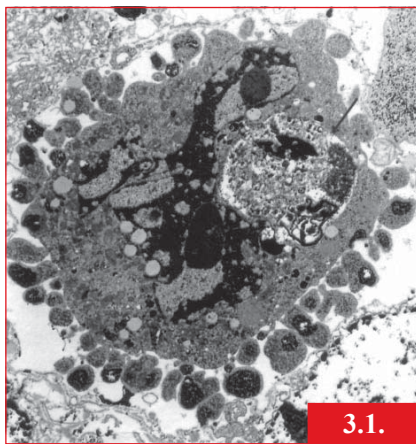
NK and immune T cells (Ly) kill tumor cells (Tc) through Ly^{FasL} →Tc^{FasR} pathway. Tc expressing FasL kill immune T cells expressing FasR: Tc^{FasL} → Ly^{FasR}. N-ras in melanoma cells down-regulates cell surface FasR expression rendering tumor cells insensitive to FasL-induced **A**. FasR⁺ melanoma cells without FasL expression transfected with FasL cDNA succumbed to endogenously induced **A**. In melanoma cells, HLA-DR signaling inhibited Fas-mediated **A**. Dacarbazine and 5-FU-treated melanoma cells became highly susceptible to perforin/granzyme-induced lysis by CTL. FasL-expressing tumor cells attract neutrophil leukocytes that can kill the tumor cells. B16F10 FasL⁺ mouse melanoma cells killed by neutrophil leukocytes induce antibody production through tumor antigen presentation by DC; the antibody through ADCC reaction protects against melanoma [328; 333-337]. TRAIL acts through receptors DR4/5 and kills susceptible cancer cells. Anti-**A** mediator FLIP protects cancer cells from TRAIL-induced **A**. Anti-FLIP siRNA restores susceptibility to **A** [338] (**1.5; 1.7; 5.4.2**)

Fas = fibroblast and spleen ♦ FADD = Fas-associated death domain ♦ DISC = death-inducing signaling domain
 ♦ TNF = tumor necrosis factor ♦ GM-CSF = granulocyte-monocyte colony stimulating factor ♦ FLIP = FLICE inhibitory protein ♦ FLICE = Fas-associated death domain-like interleukin-1-converting enzyme ♦ ADCC = antibody-dependent cellular cytotoxicity ♦ TRAIL = TNF-related apoptosis-inducing ligand ♦ siRNA = small interfering RNA ♦ DR = HLA-DR genotype

primordial cell membrane permeating and pore forming proteins. Countermeasures evolved to such cationic antimicrobial peptides (CAMP), such as changed cell envelope molecules, intracellular CAMP-trapping proteins and extrusion of CAMPs by energy-dependent efflux pumps. [314-316]. These pumps were the ancestors of multidrug-resistance efflux pumps of eukaryotes, best known to operate in chemotherapy-resistant cancer cells. Viral pathogens of protozoa (and amoebocytes) were simply excluded from intracellular entry by non-expression of their cell surface receptors. For its internal ribosome entry site (IRES), the virus uses some of its untranslated and capsid-coding nucleotides [317, 318].

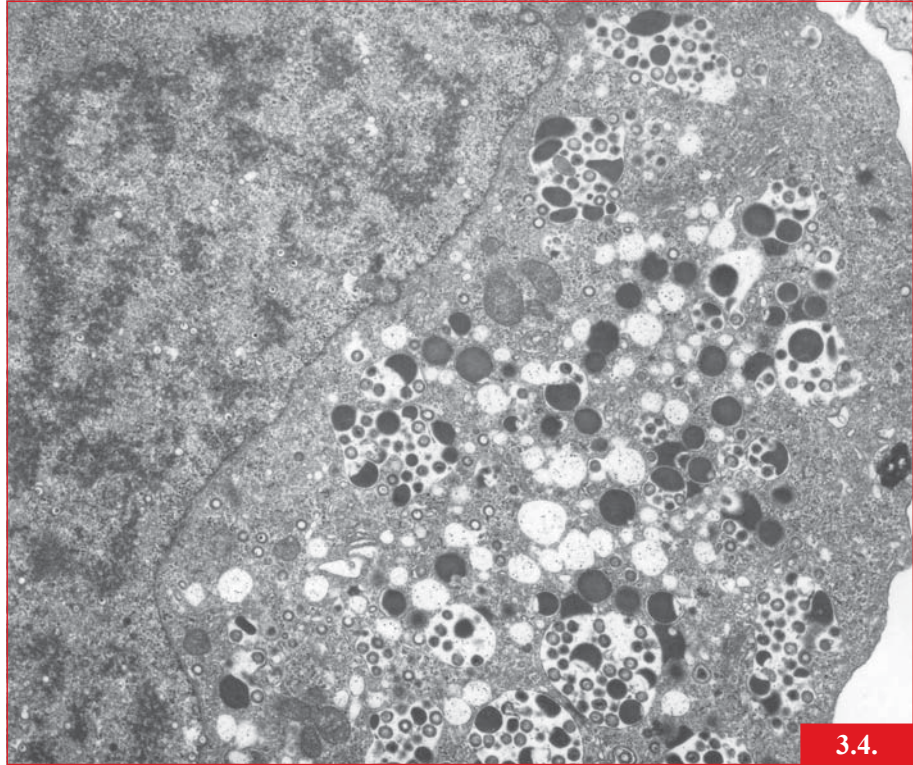
At an early time, organized cell communities forced their constituents to give up the immortality of single-celled life forms. Voluntary, intrinsic, mitochondria-initiated (cytochrome c, and caspases), and externally executed by ligand-to-receptor mechanisms (TNF to TNF-R; FasL-to-Fas; programmed death-1 receptor and its ligand, PD-1 and B7-H1/PD-L1/CD274) of death domain activations developed. The union of PD-1 with its ligands (B7-H1/PD-L1/2) suppresses the T cell receptor (TCR), so that immune T cells do not respond to antigenic stimuli with clonal expansion and cytokine production.

Tumor cells expressing these ligands paralyze immune T cells. Further, tumor cells developed **anti-apoptotic systems**: nuclear factor kappa B (NFκB); B cell lymphoma (Bcl-2), Bcl_{XL}, and related proteins; GAAP (in science: Golgi anti-apoptotic protein; in American business: generally accepted accounting principles), “life guard” proteins, like survivin) emerged. These systems had been installed very early. The anti-Bcl-2 pro-apoptotic Bax (BAX) is functional in yeast and induces autophagy [319]. The mammalian (mouse) Bax protein induces apoptotic death of plant (*Arabidopsis*) cells, except, the plant protein arabadopsis Bax inhibitor-1 (ABI-1) protects both yeast and arabadopsis cells against apoptotic death. Bax-induced apoptosis overcomes the anti-apoptotic human life guard proteins. Anti-c-GAAP (Golgi anti-apoptosis protein) (*vide infra*) siRNA induces apoptotic cell death [320].



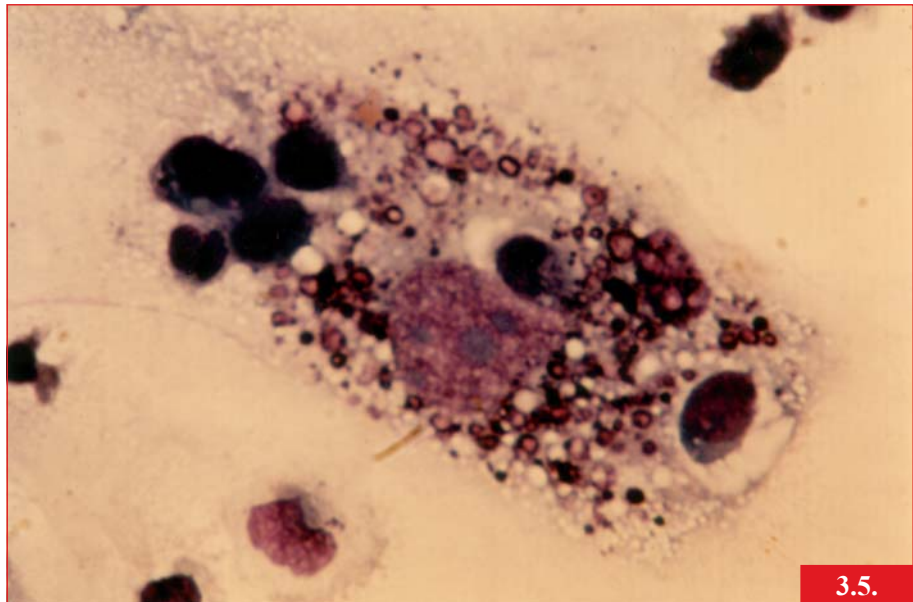
Figures (Plate) 3. 3.1. A macrophage phagocytosed and digested a lymphocyte (a lymphoma cell); the macrophage shows signs of apoptotic death: nuclear clumping and blebs (“boiling”) of its cytoplasm. Explanted human tumor cells (3.2 = ovarian carcinoma; 3.3 = rhabdomyosarcoma) dying in tissue culture with nuclear clumping and nucleolysis, cytoplasmic lysis, and cell membrane “boiling with blebs” (permit applied for re-publication) [110]. This form of cell death was not recognized as “apoptosis” in 1967. The photographer’s merit is that he focused on the cell dying in an unusual way referred to as the “death throes of a cancer cell;” “végvonaglás” in Hungarian). (The author is especially grateful to Professor Demetrios Spandidos for his permission to re-print these figures [110]. and other figures published previously in the *International Journal of Oncology*).

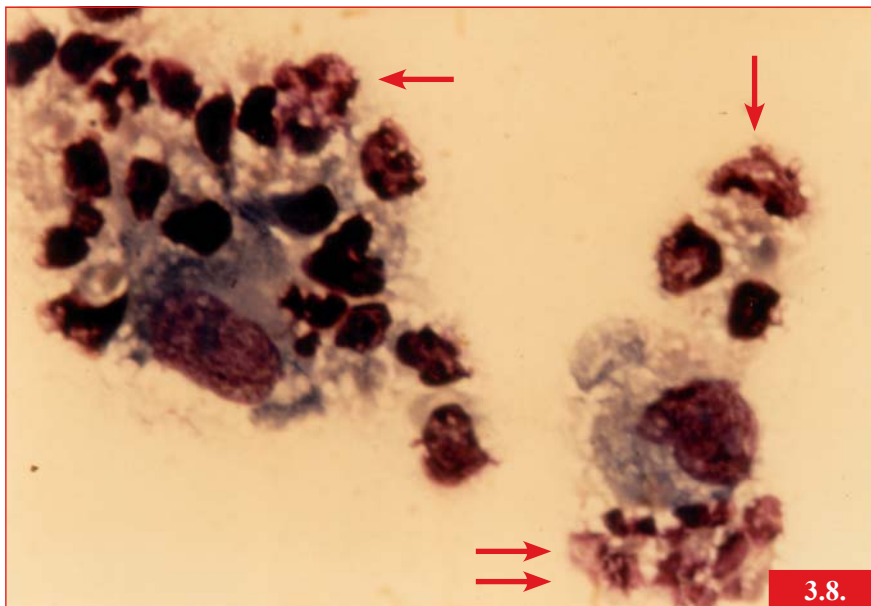
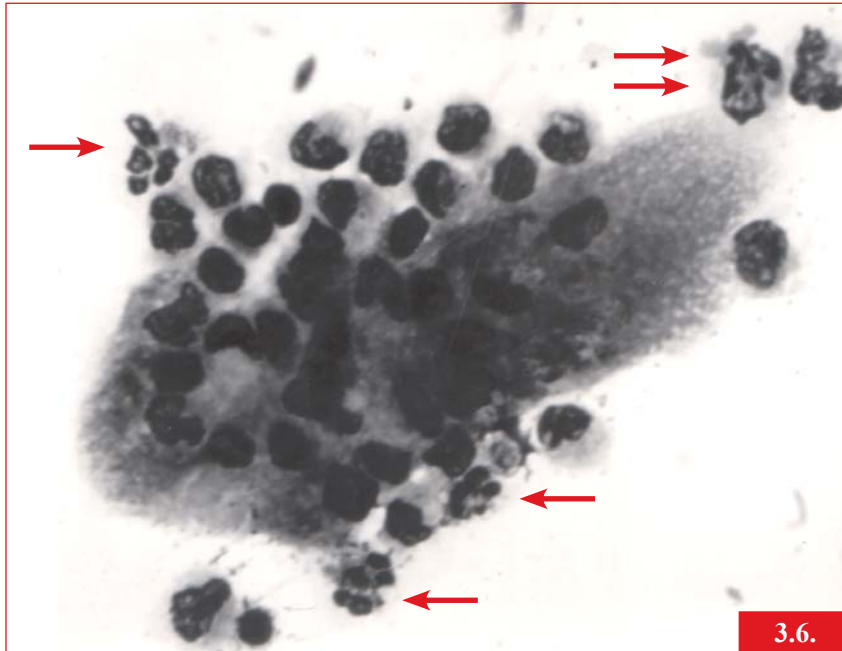
3.4. The phenomenon of autophagy in the cytoplasm of a sarcoma cell (transmission electron microscopy, unpublished).



3.5. A rhabdomyosarcoma cell of cell line #1449 [2174] shows signs of autophagy under attack by autologous small compact (later: immune T cells) lymphocytes.

(The first chondro- and rhabdomyosarcoma reports by J Sinkovics *et al* in the new *Journal of Medicine* edited by J. L. Ambrus Sr: *J Med* 1:15-25 & 313-326 1970. The author is very grateful to Professor Julian Ambrus Sr for his permission to reprint these and other figures previously published in the *Journal of Medicine*, P.J.D. Biomedical Publisher, Westbury, N.Y.).





3.6. Death by nuclear clumping (arrows) of patient's lymphocytes (N. M. MDAH#73587) attacking an autologous human chondrosarcoma cell from cell line #1459 [2174], which withstand the attack without nuclear clumping or cytolysis. Erratum: this tumor cell was once referred to as "a soft tissue sarcoma cell, like extra-osseous chondrosarcoma," [305], but was identified firmly as a chondrosarcoma cell from cell line #1459 [306; 928]. 3.7. A "counterattack by the tumor": small compact lymphocytes (immune T cells) of the patient (J.S. MDAH#82358) with fibrosarcoma attempt at attacking a cell from fibrosarcoma cell line #2113 [2174], but fail, because they, the lymphocytes, not the tumor cells, undergo nuclear clumping (arrows). 3.8. Difficult to identify human sarcoma cells [254, 306, 928] preserve their tinctorial integrity under attack by a mixed population of small compact and large granular lymphocytes; some of the lymphocytes disintegrate (arrows) under counterattack by the sarcoma cell [110; 306]. Observed and photographed in the 1970s [110, 254, 305, 306, 928], but not recognized as apoptosis at that time (**Table 4**) (**1.7**). (Applied for permission to re-publish).