Gabriele Halwachs-Baumann *Editor*

Congenital Cytomegalovirus Infection

Epidemiology, Diagnosis, Therapy

Second Edition

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Dedicated to all those people having been congenitally CMV infected because of lack of awareness, lack of diagnostic methods and lack of therapeutic possibilities

Preface

Congenital cytomegalovirus (CMV) infection is the most common intrauterine transmitted viral infection, with a tremendous impact on fetuses and newborns.

In this book, the history of this disease, its pathophysiological background, epidemiology and symptoms, as well as diagnostic and therapeutic strategies are discussed. Starting with an outline of the historical background (Chap. 1—*Long known. Long ignored*), Chaps. 2–5 are dedicated to the topics of virus host interaction for defense and transmission, epidemiology (and the influence of socioeconomic differences), diagnosis, and clinical outcome, respectively. Strategies for disease prevention and therapy are delineated in Chap. 6.

Since economic aspects are gaining more and more importance in health politics, Chap. 7 (written by E. Walter, C. Brennig, and V. Schöllbauer) is dedicated to this issue in the context of congenital CMV infection.

This work is based on the latest scientific findings and written in an understandable manner, allowing persons not working in the field of congenital CMV to also profit from it. Thus, the content is of interest not only to medical doctors, nurses, midwives, and economists, but also to a wider audience, i.e., all those who want to inform themselves about this topic. In this sense, it should not only help toward a better understanding of, but also stimulate further research on, congenital cytomegalovirus infection.

Steyr, Austria
July 2018

Gabriele Halwachs-Baumann

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Long Known, Long Ignored: A Brief History of Cytomegalovirus Research

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Gabriele Halwachs-Baumann

1.1 Beginnings: 1881–1914

In 1881, the year when president James Garfield was shot, and thereafter Chester A. Arthur was inaugurated as the president of the United States of America, in Göttingen, Germany, Professor Ribbert had to investigate the body of a syphilitic stillborn. At that time Queen Victoria ruled the British Empire. Women of the society had to wear stays and skirts covering their ankles, and it was indecorous for a gynaecologist to see the undraped alvus of a women. The examination of the small body bothered Professor Ribbert. In the kidney he found unusual large cells he could not classify [1]. More than 20 years later, in 1904, the two physicians Jesionek and Kiolemenoglou from the Royal Dermatological Hospital in Munich published a case study about 'Findings of protozoan like structures in the organs of an inherited infected luetic fetus'. They wrote that they had investigated almost all organs of the fetus, and in five of them, they found besides changes due to the infection with *Treponema pallidum* the causative organism for syphilis, idiosyncratic cellular formations, whose interpretation made great difficulties. The organs, which showed these peculiar changes, were the two kidneys, the two lungs and the liver, where clusters of 10–40 'elements' were observed. These 'elements' were 20 to 30 μm in diameter, the nuclei were large and eccentrically placed and each contained a 'central nuclear body' surrounded by two zones, a darker inner zone and a clear outer zone, which could be clearly differentiated. This accurate depiction of histological changes, taking more than two pages, is one of the most remarkable examples of exact observations in natural science [2]. Although the authors could not interpret their findings correctly, they described the 'owl eye cells', typical changes in cytomegalovirus infection, which were used as a diagnostic tool until recently.

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One has to keep in mind 100 years ago, to take a 'virus' as pathogenic agents was not granted. Only a few years before, between 1886 and 1898, the first evidence for the existence of pathogens, smaller than bacteria, was provided. Adolf Mayer from the Netherlands showed in 1882 the transmission of the mosaic illness to healthy tobacco plants by inoculating them with the sap of diseased plants. In 1892 the Russian biologist Dimitri I. Iwanowsky showed that this illness is also transmitted even when the sap was filtered by a so-called Chamberland filter, which holds off particles from the size of bacteria. M. W. Beijerinck, a Dutch soil microbiologist, confirmed Ivanowsky's observations and further showed that the infectivity of sap remained constant during serial infections of plants, providing evidence that the agent could not be a toxin, since it was able to replicate itself in living organisms [3]. In 1898 Friedrich Löffler and Paul Frosch, both students of Robert Koch, described the cause of the foot-and-mouth disease to be a particle, smaller than a bacteria, and not a liquid. They made serial transmission of filtered vesicle from diseased animals and concluded if the cause of the foot-and-mouth disease was toxin-based, then after several animal-to-animal transmissions, the original material would be so diluted that only '1:2 ½ trillion' of the starting substance would remain. 'A toxic effect of that nature would be unbelievable' they concluded. So the causative agents must be capable of reproducing itself [3–5]. In his 1899 published paper, 'a *contagium vivum fluidum* as the cause of the mosaic disease of tobacco leaves' Beijerinck used first the term 'virus' for the described infectious agents [6]. 'Virus' from the Latin word for 'poisson', first used by Aulus Cornelius Celsus (25 B.C.–about 50 AD) for the saliva of rabid dogs, became now the term for the small infectious particles, which are not bacteria but can reproduce themselves in living organisms.

So one has to excuse Jesionek and Kiolemenoglou for their assumption that the structures they found are due to protozoans, concretely *Gregarinida*, as they wrote. And Ribbert, who published his observations after he read the paper of Jesionek and Kiolemenoglou, subscribed to their view, although in the last sentence of his paper he wrote 'the value of my memorandum is mainly, by compounding the impression of those both authors, to request further investigations'. These investigations were done, and substantial difference of opinion existed among the various observers. Amoebas, coccidian and sporozoa were regarded as the source and nature of these unusual cellular formations [7]. In 1914 Smith and Weidman described similar findings and gave the name *Endameba mortinatalium* to the structures.

1.2 Between the Wars: 1914–1930

The scientific investigations in this field were adjourned by the First World War, which changed the political, social and economic structure not only of Europe but of the majority of the world. It lasted until 1921, when Ernest W. Goodpasture, at that time assistant professor at the Department of Pathology and Cancer Commission of Harvard University and better known as the first who described a rare case characterized by glomerulonephritis and haemorrhaging of the lung (Goodpasture

syndrome), in cooperation with his colleague Fritz B. Talbot, gave the observed 'protozoan-like' changes a name, by writing that '. . .it seems advisable to identify the condition with a descriptive name, and we would suggest, that it be called cytomegalia infantum'. [8] Besides this nomenclature, valid until now, they made in this paper another remarkable assumption, when they wrote that the observed cellular alterations in 'cytomegalia infantum' are similar to the skin lesions in varicella described by Tyzzer 1906 [9] and might be therefore due to the indirect effect of a similar agent on the cell. It is remarkable that Tyzzer, who was sent to the Philippines in 1904 to study the susceptibility of monkeys to smallpox (at that time varicella and variola were thought to be the minor and the major form of the same illness caused by a protozoan parasite), could study the evolution of cutaneous lesions by histopathologic examination of serial biopsies in 38 subjects, infected with varicella during an outbreak of this disease in Bilibid Prison. In the summary of his report he wrote that '. . .no important evidence has been found in favour of the hypothesis that they (the inclusions) are parasitic organisms'. So, looking back to the paper of Tyzzer, Goodpasture and Talbot were the first who supposed that the cytomegalic changes are not due to protozoan, and as it was shown later on, both infectious diseases (varicella and cytomegalia) are caused by virus of the same family.

In the same year (1921), when Goodpasture and Talbot published their paper, Benjamin Lipschütz, an Austrian dermatologist and bacteriologist, reported that similar inclusions were associated with lesions in humans and rabbits infected with herpes simplex. He maintained that the bodies or structures seen within the nucleus represent a specific reaction of the cells to a living virus. The bodies are not considered to be masses of parasites but are held to represent reaction products, associated with which is the virus, he postulated [10]. This conception of Lipschütz was not universally accepted, however. A. Luger and E. Lauda, both scientists working at the University Clinics for Internal Medicine in Vienna at the same time as Lipschütz, presumed that the 'inclusion bodies' are the result of a non-specific type of nuclear degeneration, which these authors call 'oxychromatic degeneration' [11]. All these papers were known by Rufus Cole, who became later on the first director of the Hospital of the Rockefeller Institute for Medical Research, and Ann Gayler Kuttner, who wrote her thesis for the PhD on bacteriophage phenomena, a subject which was very popular at that time ("Arrowsmith" a novel published 1925 by Sinclair Lewis, the first American to be awarded the Nobel Prize for Literature, deals with this subject). In their 1926 published paper, Cole and Kuttner gave further experimental evidence to confirm the viral aetiology of this disease, named *cytomegalia infantum* [12]. These researchers induced the production of cells containing nuclear inclusion bodies, as they are seen in herpes simplex and related conditions, by injecting material from infected submaxillary glands of guinea pigs, first filtered through a Berkefeld N filter, which was impermeable to bacteria, into the brain of anesthetized guinea pigs, less than 1 month old. They concluded therefore that the infective agent belongs to the group of filterable viruses [12].

1.3 From 1930 to 1960

Sidney Faber and S. Burt Wolbach [13] from the Department of Pathology, Harvard Medical School, and the Pathology Laboratory of the Children's Hospital, Boston, summarized in 1932 in their paper the reports on intranuclear and cytoplasmic inclusions published until that. They noted that the distribution of the inclusions in the various organs of the reported instances was as follows:

Kidneys	11 cases
Parotids	10
Lungs	8
Liver	8
Pancreas	2
Thyroid	3
Intestine	1
Sublingual gland	1
Epididymis	1

In a table they included, they listed not only the authors and the location of the inclusion bodies but the year of publication, the pathological diagnosis and the interpretation of the findings. Chronologically, beginning 1904 with Jesionek and Kiolemenoglou, 'gregarines', 'amebae or sporozoa', 'coccidian', 'embryonic epithelial cells', 'endameba mortinatalium', 'peculiar epithelial degeneration', 'abnormal cytomorphosis "cytomegalia"', 'cellular degeneration', 'filterable virus' (Von Glahn and Pappenheimer), again 'protozoa' (Walz 1926) and, last but not least, 'undecided' (Wagner 1930) were listed under the heading 'Interpretation'. Faber and Wolbach themselves removed the submaxillary glands in a series of 183 postmortem examinations of infants and found intranuclear and cytoplasmic inclusion bodies in 22 cases (12%). This was the first indication that the infection by cytomegalovirus is highly frequent. In their summary they concluded that '. . .clinical and pathological studies of the series reported reveal no association with any distinctive feature or group of symptoms or disease changes. . .there are no distinctive clinical or pathological features which would permit its recognition on the wards or in the pathology laboratory'. This heterogeneous symptomatic, where almost every organ can be involved, and the pathology can vary from mild to almost life-threatening, is still a problem in diagnosis of cytomegalovirus disease [13].

By 1932, 25 cases of a rare lethal congenital infection characterized by petechiae, hepatosplenomegaly and intracerebral calcification had been described [14]. All of them had cells with typical intranuclear inclusions. The next two decades were dominated by the Great Depression and the Second World War. Mankind had other problems than cytomegalia in neonates and toddlers. The next step forward in the research of cytomegalia was done in the 1950s. This decade was coined by the Cold War, McCarthy, Marilyn Monroe and Elvis Presley. Concerning cytomegalia John P. Wyatt and his colleagues [15] formed for this disease in 1950 the term 'generalized cytomegalic inclusion disease' (CID). Since uniform sites of

involvement were cells of the renal tubulus, they suggested that the disease might be diagnosed during life by searching for cells with inclusions in urinary sediments. Following this clue, Fetterman [16] made a cytologic preparation from 0.5 ml of urine obtained from a 3-day-old premature infant admitted to the Children's Hospital in Pittsburgh with jaundice, purpura, hepatosplenomegaly and intracerebral calcifications. He found several enormously hypertrophied cells with large intranuclear inclusions [16]. This was the first time diagnosis of cytomegalia could be done intravitum. The patient died at 4 days of age, and typical inclusions were found in the brain, pituitary, thyroid, lungs, liver and pancreas, in addition to the kidney, confirming the intravitum diagnosis of CID. W.H. Minder [17] reported in 1953 the results of electron microscope observations of pancreatic cells of a premature infant, who died 14 days after birth of CID, showing virus-like particles with a diameter of 199 nm in the nuclei and cytoplasm of infected cells. Although this was perhaps the first time the virus was seen, electron microscopy is hardly used for routine diagnostic, least of all in the 1950s of the last century. So, the technique of Fetterman, crude though it was, was better than no diagnostic technique at all. It was used with varying degrees of success for a number of years until the causative agent of the human disease was finally isolated [18].

The next milestone in the investigation of cytomegalovirus was the establishment of routinely growth of human cells in culture. The research on cytomegalovirus gained a great benefit from the work on poliovirus. John F. Enders, Frederick C. Robbins and Thomas H. Weller received the Nobel Prize in 1954 for this achievement. One year later, in 1955, Margaret Gladys Smith isolated from the submaxillary salivary gland of a 7-month-old infant dying of adrenal cortical carcinoma a virus which grew only in human but not in mouse cell culture. The paper describing this finding was rejected because she was also working with the mouse salivary gland virus and the editor thought her human agent might have been a mouse contaminant [14]. It is now known that cytomegalovirus is species specific and Margaret G. Smith was right and the editor was wrong.

Such misjudgements could occur nowadays as well; young scientists reading these words should learn from Margaret G. Smith not to lose courage but believe in their own work. It was only in 1956 when she re-isolated the virus and isolated the same virus from the kidney of a 1-month-old infant dying of generalized CID that her paper was accepted [19]. The changes she observed in the human fibroblast culture 4–7 days after oculation consisted of a few small, round or oval foci containing enlarged cells that were refractile, in contrast to normal fibroblasts. The lesions increased slowly in number and size. The centres of the lesions degenerated thereafter, leaving masses of dense, refractile granules. In fixed and stained preparations, large intranuclear inclusions were observed. Their shape usually corresponded closely to that of a nucleus. A clear, distinct zone separated the inclusion from nuclear membrane. Thus, the cytopathic changes closely resembled those seen in infected human tissues of patients with CID [7].

At the same time, Wallace P. Rowe and his co-workers in Bethesda studying the new group of adenoviruses by culturing adenoidal tissue observed an unusual type of cytopathology in the culture of adenoids from three children having tonsillectomy.

The cells in several tube cultures of each adenoid had spontaneous degeneration, characteristic of adenovirus infection, within 22 to 51 days. In one culture of each set, however, focal areas typical of a CMV infection developed after 34, 64 and 71 days of cultivation, respectively. The cytopathic changes resembled those observed by Smith. The isolated virus strain is still used as the AD169 (abbreviated from 'adenoid degeneration agent') laboratory strain of CMV [20, 21]. Contemporaneously in Boston Thomas H. Weller attempted to isolate *Toxoplasma gondii* in cell cultures. This protozoan causes a lethal congenital disease in neonates remarkably similar to CID clinically. From the liver biopsy of a 3-month-old infant with clinical signs suspected of toxoplasmosis, they wanted to isolate this infective agent. Nevertheless, the attempts to isolate *Toxoplasma* in roller cultures of human embryonic skin-muscle tissue were unsuccessful. Instead of this, the cultures showed foci of swollen cells after 12 days. Stained preparations showed cytopathology now associated with the salivary gland virus infection. The isolate is the now as Davis strain known cytomegalovirus strain [22].

Thomas H. Weller described this time in his paper published 1970 as follows [23]:

> The concurrent observation that poliomyelitis virus would grow in the skin-muscle suspended-cell system prepared for the varicella experiments brought many visitors to our laboratory. Among those, in May 1951, was Dr. Margaret Smith, who wished to apply the new methodology to the growth of the salivary gland viruses. … by 1954 (she) had accomplished her objective of isolating and serially propagating human salivary gland virus from post-mortem materials. In contrast to the considered approach of Dr. Smith in St. Louis, the initial isolations of virus in Boston and in Bethesda were serendipitous. … (In Boston) roller cultures of human embryonic skin-muscle tissue inoculated with ground liver tissue (of a 3-month-old infant with the "classical triad" of signs of congenital toxoplasmosis) did not yield Toxoplasma, but instead after 12 days showed foci of swollen cells. When stained, these foci revealed the intranuclear inclusions and cytopathology now associated with the cytomegaloviruses. … (In Bethesda) in 1955, Rowe and co-workers were recovering a new group of viruses—the adenoviruses—by observing cytopathic changes in uninoculated cultures of human adenoidal tissue. Cultures of adenoids from 3 children developed unique changes that differed from those observed with the adenoviruses. … The cytopathic changes resembled those we had described for varicella. Therefore, in May 1955, Rowe brought primary cultures of AD 169, set up on February 28, 1955, to Boston for study. … As a result of Dr. Rowe's visit, strains of virus were exchanged, and the similarity of agents recovered in St. Louis, Boston, and Bethesda was established in advance of publication.

Although nowadays excellent collaboration in science exists too, this commendable cooperation should not be forgotten.

The propagation and isolation of the virus in cell cultures, showing the aetiology of CID, and the diagnostic tool Fetterman described, lead to a growing interest in this disease. Robert D. Mercer, Sarah Luse and Donald H. Guyton from Cleveland were the first who described a case of generalized cytomegalic inclusion disease in which the diagnosis was established during the life of the patient [24]. The patient died 5 weeks after admission. A. M. Margileth from the Department of Pediatrics, US Naval Hospital, Corona, California, was one of the first who described diagnosis and

therapy of an infected newborn who survived. The examination of the microcephalic child at 14 months of age showed retardation in development. The patient was unable to sit alone, and moderate spasticity of the left hand and arm was noted. The treatment suggested was the administration of cortisone and gamma globulin [25]. Margileth concluded optimistically that '...we now have methods of diagnosing and treating cytomegalic inclusion disease of the newborn'. He did not know that he and other scientists working in this field did see at that time only the tip of the iceberg. And he did also not know that half a century later, there is still discussion of screening, diagnosis and therapy of congenital CMV infection.

The increasing interest in cytomegalic inclusion disease is also reflected by the amount of papers listed in the scientific database. From the 1940s to 1950s, the number of reports dealing with this disease rose from 2 articles from 1940 to 1950 to 96 articles from 1950 to 1960.

1.4 From 1960 to the Present

A further increase of publications was seen in the 1960s. From 1961–1970 almost 320 papers with the subject 'congenital CMV' were published. During this exciting, turbulent and revolutionary time of great social and technological changes, great effort was made in the CMV research. The isolation of the virus in tissue culture led to the development of antigens for use in a variety of serologic test. First data on epidemiology were collected leading to the statement that nonfatal cytomegalic inclusion disease in the neonatal period is a more common entity than has heretofore been appreciated. It was also speculated that congenital cytomegalic inclusion disease is seen more frequently than congenital toxoplasmosis [26]. It was supposed that some 1% of newborn infants enter the world with an active infection as indicated by the presence of viruria [27]. Early in this 'epidemiologic period' of cytomegalovirus research, social impacts were suspected to influence the occurrence of primary infections in mothers. Stern reported on serological studies showing that primary CMV infection was twice as high in immigrant Asian women compared to native-born English women [28]. In a discussion between experts written down in 1972, Hanshaw and Dudgeon presumed that there might be 4000 or more cases of congenital CMV infection per year in the United Kingdom, compared to about 200 cases per year of congenital defects due to rubella and about 30–50 and 35 cases of toxoplasmosis and congenital syphilis, respectively [28]. Although the scientific community was aware of the importance of this disease, there was a shift of interest to the problem of cytomegalovirus as fatal complication after organ transplantation. In the context of transplantation (and HIV infection later on), more sophisticated diagnostic tools were developed, assays based on molecular biology allowed new insights and CMV-specific virostatic drugs were introduced to the clinicians. These changes led to a better understanding of congenital CMV too. Nevertheless, it seems that many clinicians working in the perinatal field still forget about congenital CMV. Wyatt astonished about this behaviour already in 1950 [15] supposed for this omission a failure to recognize its importance (since it is largely a

'pathologist' disease, or a 'paediatrician' disease, by all means a disease bothering the others). Second, precariousness in interpreting diagnostic test and helplessness in choosing the right therapeutic strategies might be the reasons for this ostrichlike policy. But now, more than 120 years after the first description of this disease, there is time to solve the problem affecting still thousands of children. There exist good preconditions to do so.

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