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The Aging Human Liver: The Weal and Woe of Evolutionary Legacy

Die alternde menschliche Leber: Wohl und Wehe des evolutionären Vermächtnisses

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ABSTRACT

Aging is characterized by the progressive decline of biological integrity and its compensatory mechanisms as well as immunological dysregulation. This goes along with an increasing risk of frailty and disease. Against this background, we here specifically focus on the aging of the human liver. For the first time, we shed light on the intertwining evolutionary underpinnings of the liver's declining regenerative capacity, the phenomenon of inflammaging, and the biotransformation capacity in the process of aging. In addition, we discuss how aging influences the risk for developing nonalcoholic fatty liver disease, hepatocellular carcinoma, and/or autoimmune hepatitis, and we describe chronic diseases as accelerators of biological aging.

ZUSAMMENFASSUNG

Altern ist gekennzeichnet durch die progrediente Abnahme der biologischen Integrität und ihrer kompensatorischen Mechanismen sowie immunologische Dysregulation. Dies geht mit einem zunehmenden Risiko für Gebrechlichkeit und Krankheit einher. Vor diesem Hintergrund fokussieren wir auf die Alterung der menschlichen Leber. Dabei beleuchten wir erstmals die ineinandergreifenden evolutiven Fundamente der abnehmenden Leberregenerationsfähigkeit, des Inflammaging-Phänomens und der Biotransformationskapazität im Alterungsprozess. Zudem diskutieren wir den Einfluss des Alterns auf das Risiko, eine nichtalkoholische Fettlebererkrankung, ein Hepatozelluläres Karzinom und/oder eine Autoimmunhepatitis zu entwickeln, und wir beschreiben chronische Erkrankungen als Beschleuniger des biologischen Alterns.

Introduction

Aging is a function of general biological deterioration of structure and function over time, combined with the decline of counteracting mechanisms of repair and regeneration. This progressive change entails an increased risk of disease and frailty, and it eventually leads to death [1]. How we age individually is influenced by both endogenous and exogenous factors – perhaps with our genetic makeup and our dietary habits as the most important contributing factors. Nevertheless, the course of aging is also considerably impacted by chronic disease, which likewise is influenced by genetic predisposition and nutrition.

Against this background, we here provide an overview of the current state of knowledge specifically on the aging of the liver as an organ whose immense range of tasks is indispensable and of altruistic value for the entire organism. In this biological context, we first focus on crucial evolutionary underpinnings of the human liver's regenerative capacity to then address hepatic aging with respect to the influence of our evolutionary heritage on the liver regeneration in age, the phenomenon of inflammaging, and

hepatic biotransformation functions affected in age. All these aspects are associated with the dwindling performance of the human liver in the elderly and its increased susceptibility to certain diseases.

Hepatic age-dependent alterations may impact the occurrence of certain chronic liver diseases and, vice versa, such chronic diseases influence liver aging. In some of these cases, there may be mutual interdependencies. We here particularly highlight the impact of hepatic aging on the development of nonalcoholic fatty liver disease (**NAFLD**) and of hepatocellular carcinoma (**HCC**). NAFLD has emerged as the most prevalent chronic liver disease. Consisting of steatotic nonalcoholic fatty liver (**NAFL**) in its earlier phase and inflammatory nonalcoholic steatohepatitis (**NASH**) as a late-stage exacerbation, this major pandemic currently affects close to 30 % of the global population [2, 3]. In addition, NAFLD is a leading cause of HCC [4], with NASH being the foremost risk factor for developing this type of cancer [5]. The incidence of HCC has increased sharply, and if only HCC is considered among all types of primary liver cancer (accounting for 75 %–85 % of all cases), it has the fifth-highest mortality rate of all cancer entities [6]. We therefore urgently need more effective means to control and contain NAFLD – not least to prevent the NASH-dependent increase in HCC cases. In this regard, understanding the evolutionary roots of those essential functions of the liver that derail with age and contribute significantly to the occurrence of NAFLD and HCC may be revealing.

Heirlooms from the Dim and Distant Past

The human liver's ability to regenerate upon insult is of outstanding importance. In contrast, medical literature not always – but far too often – neglects the evolutionary context of this fascinating capacity. Such negligence entails the risk of overlooking crucial information. It is thus essential to expand on this topic, as age-related changes in liver regeneration should be considered against their basic evolutionary background. To this end, we here repeatedly (though not exclusively) refer to Delgado-Coello, whose recent review article [7] served admirably to bring this topic to the fore. In mammals, liver regeneration is realized by a combined increase in cell size (hypertrophy) and in cell numbers (hyperplasia). The latter is usually dominant [7], and it is now widely accepted that, what still is mostly referred to as 'liver regeneration' is more accurately reflected by the term 'hepatic compensatory hyperplasia' [8]. This terminology also refers to the fact that the liver does not regain its original shape in the course of this process [9]. Importantly, compensatory hyperplasia differs from the biological regeneration process that can replace lost appendages in animals having this capacity [10].

Invertebrates and vertebrates split from a common ancestor roughly 550 million years ago [11]. This decisive evolutionary event was followed by a series of key steps that, over prolonged periods of time, led to the emergence of the vertebrate liver and subsequently realized its complex regenerative capacity in higher vertebrates. In ascending order, the subphylum of vertebrates comprises five classes of ectotherms/poikilotherms (fishes, amphibians and reptiles) and endotherms/homoiotherms (birds and mammals). Current knowledge, although still incomplete, suggests that the increase in complexity of the process of compensatory liver hyperplasia likewise progressed along this sequence [7]. Conversely however, while invertebrates usually show a remarkable ability to regenerate organs and appendages, this ability progressively declined as evolution advanced towards complex vertebrates [12]. Thus, the highest level of hepatic regenerative capacity has been achieved in mammals, including ourselves [7, 12]. Consequently, the regenerative capacity of the whole individual has declined over time in favor of the liver's ever-improving regenerative capacity.

Several factors are discussed as potential reasons for this inverse development: Regarding the gradual loss of the general regeneration capacity, these factors include (i) the evolutionary gain/loss of regeneration-associated genes and molecular machinery (which, however, seems more like taking an inventory rather than offering a genuine explanatory approach); (ii) the phylogenetic ectotherm-to-endotherm transition; and (iii) *nota bene*, the continuous improvement of the immune system throughout evolution [12]. Amazingly, the latter also has been a major driving force for the development of the liver's specific regenerative capacity. Of course, no highly interconnected networks of immune cells as we know them from much more advanced evolutionary levels were at that time in existence – but during this early period the first signaling molecules evolved that later assumed key functions in both immunity and hepatic regeneration. Also, as discussed further below, these factors provide insightful cross-connections to the phenomenon of inflammaging. It thus may be fair to say that the pillars of both immunity and liver regeneration were erected in those early days.

All of these signals highly relevant in regulating hepatic regeneration evolved at least 550 million years ago [7]. Thus, we come full circle with the separation of invertebrates and vertebrates from their common ancestor mentioned above. In other words, the biological decision-making processes that ultimately evolved the remarkable regenerative capacity of the human liver as well as key immune mechanisms – with both of which being increasingly corrupted as the liver ages (see below) – can be traced back to this primordial root event. Therefore, such valuable heirlooms from the Dim and Distant Past are among the foundations of present life. It should hardly surprise that exactly the same essential functions – or, rather, their gradual regulatory derailment – are of outstanding importance in aging as well.

At this point, let us quote Delgado-Coello, who aptly summarized important facts and well-founded assumptions: *The toolkit comprising a diversity of transcription factors undoubtedly evolved in parallel with the origin and evolution of metazoans (…). Some of the mechanisms for production of new cells in order to regenerate any kind of tissue are dedifferentiation (…), transdifferentiation (…), and activation of pools of different stem cells (…). Since these mechanisms are not present in the majority of adult mammals, they show a low regenerative ability. However, it seems that given the longer life span of vertebrates in comparison to lower species, they were supplied in the course of evolution with different mechanisms for the repair of tissues, such as liver regeneration to preserve integrity (…)* [7] (for references omitted from this quotation, see the original article). Against this background, Delgado-Coello further agreed with

Cox and Goessling to the effect that hepatic compensatory hyperplasia is an adaptive response to liver injury [7, 12], and she importantly pointed out that the excessive production of extracellular matrix (**ECM**) and scarring/fibrosis upon chronic impairment must be considered a maladaptive response that undermines the liver's effective regeneration [7]. In this respect, it should be noted that fibrosis is the result of a bidirectional process – i. e., the dynamic interplay of fibrogenesis and fibrolysis – with the latter leading to the regression of fibrosis once the underlying cause is resolved [13]. Therefore, excessive fibrosis can result from an imbalance of these counteracting mechanisms.

Also in context with the topic of this Review, specific reasons correlating with the individual life expectancy are of great interest as some of these may even apply to singular organs such as the liver. In this regard, a long-standing theory by Sir Peter Brian Medawar stands out: To a wider audience, Medawar is known for sharing a Nobel Prize in 1960 for discovering acquired immunological tolerance [14]. Besides, however, his interest in genetics led him to formulate a mutation accumulation theory of aging (which he referred to as 'senescence'), wherein he suggested aging to result from the gradually declining force of natural selection. His theory proposed highly expressed genes in old adults to be under a weaker selection pressure than in younger adults [15]. Sixty-seven years later, this prediction was confirmed by Turan et al. who found an age-related decrease in hepatic transcriptome conservation [16]. In stark contrast, however, very long-lived individuals – i. e., centenarians and supercentenarians – differ from those with average lifespans in that their expressions of inflammaging- and senescence-related genes remain tightly controlled despite their advanced age [17]. This remarkable distinction also applies to hepatic aging: Barth et al. found that most differentially expressed genes in the aging of the liver belong to immunological and inflammatory processes. Their data further imply that apoptosis plays a more important role during hepatic aging than in any of the other investigated tissues (blood, brain, and skin), and long-lived individuals also appear to have a more active or better regulated response protecting from reactive oxygen species [17]. However, we must clearly admit that, at present, findings such as these do not enable clear conclusions on their clinical relevance in age. Still, they suggest that the consistently more stable expression of certain genes in (super)centenarians at least contributes to longevity. Accordingly, potential therapeutic applications that could result from such findings are already discussed in the respective publications.

As for the 'weal and woe of evolutionary legacy' alluded to in the title of this article, the evolutionary mechanisms deeply embedded in our biological memory are prime guiding principles that render excellent services to our well-being throughout a long period of our individual lifetimes. However, their successive decline after the onset of aging has profound consequences: due to their essential nature, they cannot be compensated for – and so their decay entails biological derailment and, eventually, individual demise.

▶ **Fig. 1** provides an overview of the above information, as well as of the associations and connections described below that arise from our evolutionary heritage for the aging human liver and the development of certain age-related liver diseases.

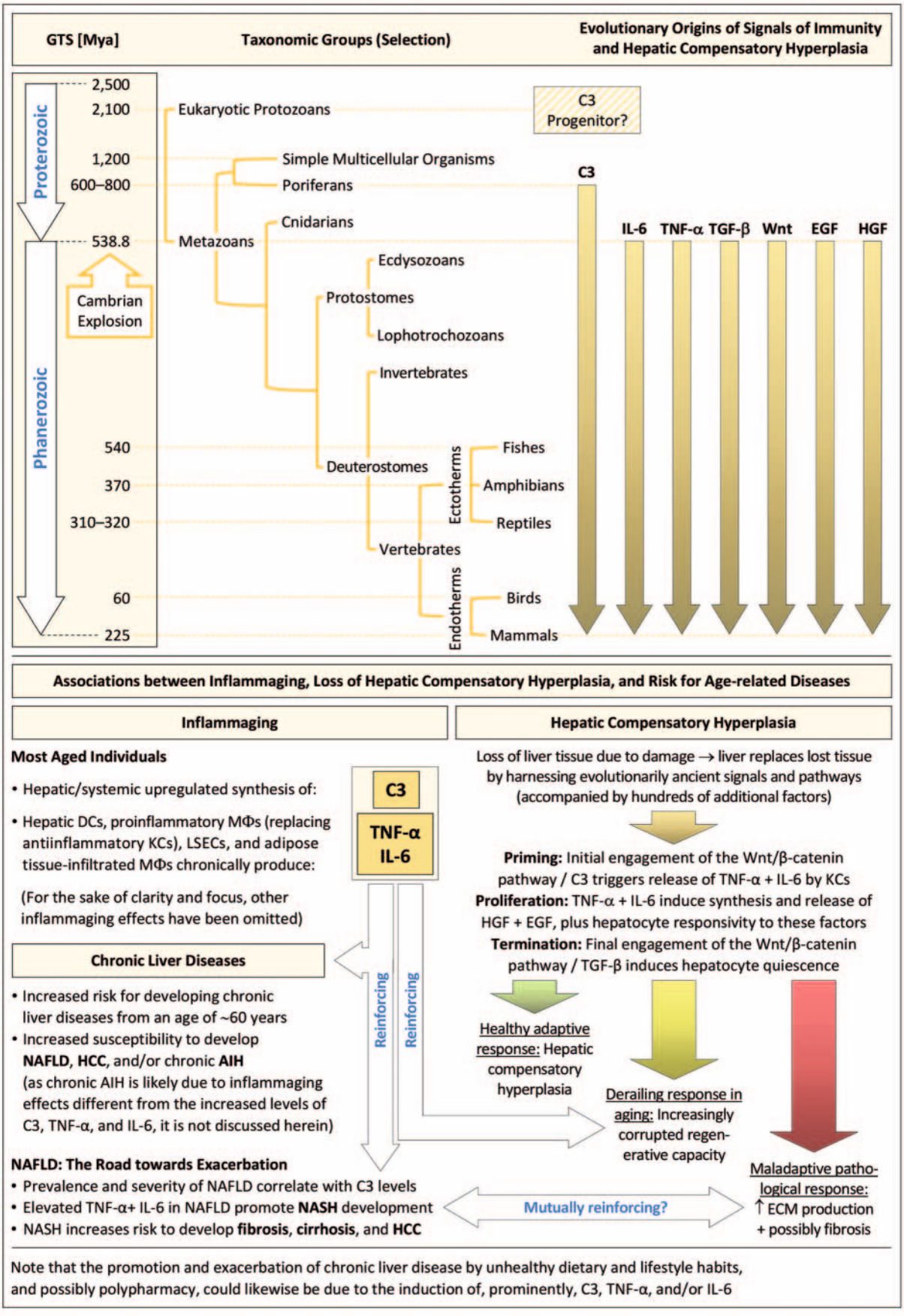
Hepatic Compensatory Hyperplasia

In humans, the liver is the only solid organ potentially capable to regenerate after tissue loss from as little as 25 % of its volume [18], as enabled by hepatocytes and cholangiocytes that can transdifferentiate into each other as facultative stem cells [19]. The 25 % figure represents an extreme lower limit, which likely is significantly higher in clinical practice. However, a greater body of reliable data on the human liver related to different clinical contexts is not yet available. From studies essentially performed in rodent models we know that age is a highly important factor. In adulthood, the liver can indeed regenerate upon partial hepatectomy or partial liver transplantation, but its regenerative capacity gradually declines with aging. In addition, factors such as, but not limited to, certain pathological processes, steatosis, metabolic demand, and long-lasting ischemia/hypoxia further influence the liver's regenerative capacity. If the delicate balance between these and other influencing factors is not adequately accounted for, acute liver failure can result [20]. So clearly, we need more data from different diseases and clinical settings.

Damage to the liver can be caused by the loss of liver tissue (see above), viral or chemical injury (i. e., infection or intoxication), or lifestyle factors (e. g., unhealthy diet, lack of exercise). In all of these cases, the liver attempts to replace the lost tissue [21]. It is therefore particularly noteworthy that the most dramatic effect of aging on the liver – first reported by Nancy Bucher and colleagues in 1964 – is the decline in this regenerative potential, as reflected in a significant delay and reduction in compensatory hepatocyte proliferation in advanced age [22]. As to potential underlying mechanisms, Timchenko had suggested a crucial role for age-dependent epigenetic silencing of the cellular proliferation potential. First evidence indicating epigenetic inhibition of liver proliferation in old mice was obtained by studies on the transcription factor C/EBPα – a is a central regulator of energy metabolism. While complexing the cyclin-dependent kinase cdk2 in young livers, C/EBPα instead establishes high-molecular-weight complexes with Rb, E2F4 and the chromatin-remodeling protein Brm in the livers of old mice. These C/EBPα–Brm complexes occupy E2F-dependent gene promoters such as b-myb, cdc2, DHFR and c-myc, which leads to their epigenetic silencing, and they further inhibit the expression of the transcription factor FoxM1B. In aged individuals, these and additional epigenetic effects compromise the liver's regenerative potential [23]. While acknowledging that epigenetic silencing thus definitely assumes a role in this context, we still argue that the following mechanisms are at least as important.

It is evident that, upon injury, the liver reactivates evolutionarily ancient developmental and differentiation pathways for enabling hepatic compensatory hyperplasia. Indeed, among the hundreds of factors expressed and activated for orchestrating this process [19], the ones that stand out most prominently are those that traveled a long evolutionary path. They are deeply embedded in a compensatory response that is divided into three phases:

The ***priming phase*** is initiated by perhaps the most ancient immunological signaling molecule, complement factor C3, which originally derived from α_2-macroglobulin. Undisputedly, sponges



▶ **Fig. 1** Associations between the evolutionary underpinnings of inflammaging, the decline of hepatic regeneration in aging, and the development of liver diseases in the elderly. **Upper half:** Evolutionary introduction of pivotal signals underlying both the innate branch of immunity and the liver's regenerative capacity. A primeval variant of complement factor C3 is the first major molecule of the immune system known to have evolved; it has been evidenced in early metazoans (poriferans) and may already have been present in more simple multicellular organisms and perhaps even protozoans. Primordial variants of IL-6, TNF-α, TGF-β, Wnt, EGF, and HGF date back to comparable periods, which collectively may imply their development in response to the Cambrian explosion – a time when most metazoan phyla first appeared in the fossil record. Also known as the 'Biological Big Bang', this event separates the geological eras of the Proterozoic and the Phanerozoic. In the course of evolution, the 'primordial factors' have evolved and branched extensively, as can be seen, for example, from the immense number of members of the mammalian tumor necrosis factor superfamily. Nevertheless, their high degree of conservation documents the indispensable biological roles of these factors. The components of adaptive immunity only arose later in vertebrates (not shown). Note that the time scale is not linear. Also note that the actually more comprehensive evolutionary relationships have been truncated to the chronological and taxonomic information relevant in the context of this article. **Lower half:** With the prominent exception of centenarians and supercentenarians, inflammaging is a hallmark of old age. Specifically, the chronic increase in the production of C3, TNF-α and IL-6 by the aging immune system reveals a striking common denominator to which other age-related inflammatory changes seem to be subordinate. The age-related chronic presence of these central factors both promotes the development of age-related (liver) diseases and corrupts the regulation of liver regeneration. At least in the case of NAFLD, both processes might amplify each other. **Abbreviations: Ag** – antigen; **AIH** – autoimmune hepatitis; **C3** – complement factor C3; **DCs** – dendritic cells; **ECM** – extracellular matrix; **EGF** – epidermal growth factor; **GTS** – Geologic Time Scale; **HCC** – hepatocellular carcinoma; **HGF** – hepatocyte growth factor; **HSCs** – hepatic stellate cells; **IL-6** – interleukin 6; **KCs** – Kupffer cells; **LSECs** – liver sinusoidal endothelial cells; **MΦs** – macrophages; **Mya** – million years ago; **NAFLD** – nonalcoholic fatty liver disease; **NASH** – nonalcoholic steatohepatitis; **TGF-β** – transforming growth factor β; **TNF-α** – tumor necrosis factor α; **Wnt** – Wingless-related integration site or Wingless/Int-1, respectively (a key component of the Wnt/β-catenin pathway).

(phylum Porifera) – the first metazoans at the root of the animal tree [24] that emerged 600–800 million years ago [25, 26] – are endowed with a precursor variant of C3. Still, an even older molecular predecessor may already have been present in protozoans [27]. In mammals, C3 acts as a very early and decisive factor in the priming phase of hepatic compensatory hyperplasia [28]. Strikingly, this does not occur via the regular pathways of complement activation [29], which indicates that the liver might utilize a primal C3 activation pathway in the context of compensatory repair, whereas the three more commonly known C3 activation pathways are more recent immunological developments. Moreover, it then turned out that C3 regulates the induction of interleukin 6 (**IL-6**) [30] and promotes tumor necrosis factor α (**TNF-α**) signaling [31] (for detail, see Markiewski et al. [32]). These findings were exciting because C3, TNF-α, and IL-6 are similarly significant players in the priming phase of hepatic regeneration and, like C3, the two latter have been around for long as well: in his pioneering study, Ahne first evidenced the presence of TNF-α and IL-6 in fish, amphibians and reptiles, which suggested that their origin dates back more than more than 400 million years [33] – a first tentative approximation later set at 550–600 million years [34, 35]. During the hepatic compensatory response, C3 specifically activates Kupffer cells to release TNF-α and IL-6 that reach high levels in the peripheral blood 2–5 hours after liver damage [36, 37].

The ***proliferative phase*** of hepatocellular regeneration is likewise dominated by ancient growth and differentiation factors – i. e., the hepatocyte and epidermal growth factors (**HGF**; **EGF**) [38]. Initial stimulation of hepatocytes with TNF-α and IL-6 (in concert with other factors) induces transcription factors NF-κB, STAT3, AP-1, and C/EBPβ that render hepatocytes fully responsive to HGF and EGF. TNF-α and IL-6 also initiate extensive remodeling of the ECM from which prestored HGF is liberated [21]. A common ancestor of HGF and other serine proteinases can be traced back more than 500 million years [39], and results from a broad genomic survey suggest that the EGF/EGF receptor pathway evolved within metazoans [40]. During liver regeneration, HGF is released from the ECM about one hour after injury [41, 42] and is also synthesized by hepatic cell populations. In contrast, EGF is produced in extrahepatic sites such as the Brunner's glands of the duodenum [43] that, interestingly, are unique to mammals [44]. Importantly, the ancient HGF- and EGF-driven signaling pathways can provide redundancy for each other, but proper functionality of at least one of them is mandatory to sustain liver regeneration [45].

Finally, transforming growth factor β (**TGF-β**) – which has been around since the emergence of metazoans [46, 47] – plays a key role in the ***termination phase*** of liver regeneration [7]. In this conclusive phase, TGF-β represses urokinase activity, inhibits hepatocyte proliferation, and induces apoptosis, which collectively resets the hepatocytes to quiescence [21, 48]. Moreover, the Wnt/β-catenin pathway (wherein **Wnt** stands for 'Wingless-related integration site' or 'Wingless/Int-1', respectively), which has remained highly conserved throughout invertebrate and vertebrate evolution [49], is involved both in ***priming*** and ***termination*** [7]. Thus, this pathway obviously serves as an 'overarching theme' in the orchestration of liver regeneration. While otherwise regulating embryonic development as well as cellular proliferation/differentiation and usually being silenced in adulthood [50], the Wnt/β-catenin pathway plays a key role as a master regulator of liver regeneration [12]. Perturbations of this central signaling pathway have profound consequences: In the liver, aberrant Wnt/β-catenin signaling promotes the onset and progression of HCC and cholangiocarcinoma [51], while continuous reactivation of this pathway can trigger accelerated cellular senescence [50].

Each of the mediators and signaling pathways mentioned are indispensable. If disrupted in the aging process, the liver's regenerative potential gradually wanes.

Inflammaging

At first glance, the effect of aging on the immune system may seem paradoxical, as certain antigen-specific immune responses decrease, while the risk for chronic inflammation and autoimmunity increases [52, 53]. However, most of these observations are

explainable by aging-related changes in the generation, maturation, and activation of different immune cell populations and their activity profiles. Specifically, while immunity against infectious agents and tumor cells diminish, aging conversely goes along with the phenomenon of inflammaging and a higher risk of developing autoimmune diseases [53]. The term inflammaging – originally coined by Franceschi and colleagues as *'inflamm-aging'* within an evolutionary perspective – captures aging-associated increases in proinflammatory processes that go along with a greater susceptibility to certain diseases in the elderly [54]. As for the aging liver, these immunological changes correlate with increased incidence rates of viral hepatitis, HCC, and autoimmune hepatitis [55]. Hallmarks of inflammaging include the chronically increased production of proinflammatory cytokines TNF-α and IL-6 by various intrahepatic immune cell populations as well as by cells of the hepatic sinusoid and of liver-associated adipocytes. Unhealthy dietary and lifestyle habits as well as polypharmacy further aggravate the degree of inflammaging. Overall, inflammaging entails frailty as well as increased disease susceptibility, morbidity, and mortality [54, 56, 57].

We now consider some cellular key components of inflammaging in detail.

Dendritic cells

As immunological masterminds, myeloid dendritic cells (**DCs**) act at the interface of the innate and adaptive branches of the immune system, and they play key roles in the maintenance of immune tolerance and the induction of primary antigen-specific immune responses [58, 59]. Their importance is underscored by the profound immunological impact of DC deficiency syndromes [60]. With age, the functionality of this major turnstile of immunity is compromised: Specifically, the DCs' ability to take up antigens (a precondition for subsequent antigen processing and presentation [59]) and to migrate from non-lymphoid to lymphoid organs (which correlates with functional maturation [59]) is impaired. This corresponds with the DCs' decreased ability to respond to foreign antigens as well as their diminished capacity to prime antigen-specific T-cell responses. On the other hand, these cells display upregulated autoreactivity, which may relate to the fact that DCs in aged individuals produce an increased 'background noise' of proinflammatory cytokines that prominently include TNF-α and IL-6 [61]. Thus, with advancing age, this central cell population exhibits significant functional changes consistent with inflammaging and the increasing incidence of certain age-related diseases.

Macrophages

Another major population of antigen-presenting cells besides DCs are macrophages (**MΦs**). Being derived from myeloid bone marrow precursors, and provided appropriate priming or polarization, they have a much higher proinflammatory potential than the DCs. Thus, MΦs can assume various proinflammatory phenotypes, and they significantly contribute to the development of non-infectious inflammatory diseases [62]. With Kupffer cells (**KCs**), however, the liver is equipped with a distinctive MΦ population: unlike the MΦ variants encountered in other organs and tissues, KCs originate from erythro-myeloid progenitors that develop in the yolk sac (refuting the earlier concept that KCs arise from bone marrow precursors [63]) and that subsequently colonize the nascent fetal liver where giving rise to KCs that renew themselves locally throughout adult life. Importantly, these peculiar MΦs usually are a quiescent and antiinflammatory population [64, 65]. This status, however, changes drastically upon aging: For example, livers of old rats reveal elevated percentages of infiltrated bone marrow-derived $CD68^+$ MΦs, which replace the KC phenotype and produce increased amounts of IL-6 [66]. Such data from liver tissue of healthy humans are not yet available, but several studies in patients with liver inflammation, fibrosis, NAFLD and/or HCC indeed demonstrated increased numbers of hepatic $CD68^+$ MΦs (e. g., [67, 68]). Future research needs to clarify whether such cells are actively involved in the onset and/or exacerbation of those conditions in age. Another influencing factor is the degree of necroptosis – an inflammation-promoting cell death mechanism – that is significantly upregulated in the aged liver. Specifically, elevated levels of necroptosis in hepatocytes and liver MΦs of old mice were paralleled by significant increases in proinflammatory MΦs, upregulated TNF-α, IL-6, and IL1-β secretion, and the occurrence of liver fibrosis. The fact that inhibiting necroptosis returned these and other aging-associated parameters close to the levels of young mice evidenced that aging-associated necroptosis contributes to inflammaging and fibrosis [69].

Sinusoidal endothelial cells and hepatic stellate cells

The specialized population of liver sinusoidal endothelial cells (**LSECs**) regulates hepatic homeostasis. Their thin morphology and fenestrations allow for substance exchange between the circulation and the liver. In age, however, the phenomenon of pseudocapillarization – i. e., a reduction in the number and size of their fenestrations – decreases the hepatic blood flow and the availability of nitric oxide. Moreover, LSECs suffer from increased oxidative stress and ramp up their production of proinflammatory cytokines. Other effects of age-related LSEC changes may include hepatic hypoxia, activation of hepatic stellate cells (**HSCs**), and liver fibrosis [70]. The latter is due to the well-established role of activated HSCs that are transdifferentiated into profibrotic myofibroblasts producing ECM components [71]; in addition, there is evidence for an involvement of bone marrow-derived myofibroblasts in the development of liver fibrosis [72].

Adipocytes of white adipose tissue

The most important environmental factor affecting life expectancy is nutrition, which at the same time has a major impact on the aging liver as the central metabolic organ [73]. In this regard, white adipose tissue (**WAT**) should be considered as an essential variable in the equation of aging-dependent changes favoring the development of chronic liver disease. Moreover, prolonged detrimental eating habits feeding adipocytes sum up over a lifetime [74] to take their toll in old age. The key task of WAT is to maintain energy homeostasis: Upon excess energy availability, it increases lipid storage and systemically communicates nutritional abundance, while releasing stored triglycerides upon energy depletion as free fatty acids to support catabolism. In aging how-

ever, the cellular composition of the different WAT depots shifts to facilitate increased inflammatory cell infiltration [75]. The resultant release of proinflammatory cytokines TNF-α and IL-6 contributes to inflammaging [76] and, in concert with oxidative stress and lipotoxicity, leads to a higher prevalence of metabolic disease in the elderly and, *inter alia*, promotes NAFLD development [77, 78, 79]. In this context, we showed that the visceral WAT depot contributes to the pathogenesis of NAFLD by displaying – within this depot – a combination of different and independently regulated cell death mechanisms (apoptosis, necrosis, necroptosis, and autophagy) as well as fibrosis [80]. Based on the increasing body of evidence on a lively organokine crosstalk in the metabolic diseases [81], we assume that these changes are communicated to the diseased liver *via* adipokine messaging.

Chronic Liver Diseases

Aging generally is a major risk factor for developing chronic disease [82, 83, 84]. In the human liver, an age of about 60 years goes along with multifactorial changes that collectively increase the risk for developing chronic diseases affecting this organ. These include the phenomenon of inflammaging and the aforementioned changes in certain hepatic cell populations. In addition, there are changes summarized under the term 'immunosenescence'; they encompass all non-inflammatory alterations of immunity that comprise shifts in the compositions of cell populations of the innate and acquired immune system as well as of their surface marker expressions, cytokine secretion patterns, and intracellular signaling pathways [82, 83]. Moreover, the aged liver strikes by various changes related to nucleic acids at the levels of genome-wide DNA methylation [85], characteristic transcriptome alterations [86], and the presence of certain microRNAs (i. e., 31-5 p, 141-3 p and 200c-3 p) that, therefore, can serve as aging markers [87]. (Since discussing these changes and aging markers in greater depth would by far exceed the scope of this evolutionarily focused Review, we refer interested readers to the references provided.) The combination of all these age-related changes results in more severe infections, impaired responses to vaccinations, and an increased risk of developing chronic, malignant, and/or autoimmune diseases [55, 88].

We now present some salient yet non-exhaustive aspects on chronic liver diseases and aging.

Nonalcoholic fatty liver disease and hepatocellular carcinoma

In NAFLD, the production of TNF-α, IL-6, and IL-1β recruits and activates KCs [89] whereby promoting the development of NASH [90, 91]. Importantly, concentrations of certain proinflammatory mediators increase with age, which applies to both TNF-α and IL-6 in non-obese patients *vs.* TNF-α only in obese patients. These increased cytokine concentrations not only affect the liver, but also result in systemic inflammation [92].

Moreover, NASH is a serious risk factor for developing liver cirrhosis and HCC. The fact that old age usually goes along with inflammaging promotes this downhill development [93]. Accordingly, HCC has a high incidence in the elderly, which indicates that advanced age is a key risk factor for developing HCC. However, current understanding is obviously insufficient, as we cannot explain why the incidence of HCC suddenly decreases markedly after an age of 70 years [93].

Autoimmune hepatitis

Generally, the increased risk for autoimmunity in the elderly might be due to the impairment of DC maturation, the decrease in regulatory T cells [53], and the increased proliferation of peripheral naive T cells in age [94]. Therefore, according to current knowledge, the age-related increase in autoimmunity is at least not directly related to the evolutionarily based factors discussed herein. Specifically as to the liver, autoimmune hepatitis may either present as acute or as chronic hepatitis. For patients suffering from chronic autoimmune hepatitis, Rizvi and Gawrieh recommended steroid-sparing or -minimizing therapeutic regimens – with budesonide or low-dose prednisone combined with azathioprine during the induction phase – while avoiding high-dose prednisone monotherapy because of the increased risk of side-effects [55].

The Horvath clock: Chronic disease accelerates biological aging

Several biomarkers allow to determine biological age more precisely than by chronological age. The most accurate epigenetic biomarker is the DNA methylation status of cytosine/guanine (**CpG**) dinucleotides [95]. Meticulous screening of the almost 30 million CpG sites in the human genome undergoing age-associated changes in methylation status led to the identification of the Horvath clock that is based on the methylation statuses of 353 distinct CpG sites of which 193 gain methylation and 160 lose methylation over time [96]. This epigenetic clock allows to precisely determine the biological age. Accordingly, while slowed down in (super)centenarians and their offspring [97], biological aging is accelerated in patients suffering from chronic diseases. These prominently include, but are not limited to, certain viral infections (presently evidenced for hepatitis B, hepatitis C, and HIV disease), various cancers, renal disease, cardiovascular disease [95] and, importantly, liver fibrosis within the context of NAFLD [98]. As a corollary of the foregoing, it may be clinically advisable to adapt medication dosages for patients suffering from chronic diseases to the respective guideline recommendations for the age group ranging directly above the patients' chronological age group to more closely match their biological age. At the same time, such practice will account for the decreased biotransformation capacity of such patients.

Biotransformation

The liver is the main site of drug detoxification. Being exposed to a variety of potentially harmful chemicals – including pharmaceuticals – can lead to cell death and injury. For many decades of an individual's life, the liver is well prepared for compensating such damage by its regenerative capacities [99], but this usually changes with age. In 1998, Schmucker stated that the reduced hepatic

clearance of certain drugs and the marked increase in the frequency of adverse drug effects in geriatric patients reflect a reduced liver volume and blood flow as well as an increase in polypharmacy regimens rather than impaired phase I metabolism [100]. However, we now know that hepatic drug metabolism mainly depends on the phase I system – and especially cytochrome P_{450} – which starts to progressively decline after the fifth decade of life. Further aspects impacting on pharmacotherapy are the decrease in body weight and renal function in age [73]. Collectively, these alterations increase the susceptibility to drug-induced liver injury in age [53], which mostly is due to antibiotics and cardiovascular drugs [101]. Therefore, elderly patients receiving such treatments should be monitored closely. It is self-evident that the combined impact of inflammaging, declining hepatic compensatory hyperplasia and, if present, additional chronic liver disease increasingly impairs the biotransformation capacity in old age.

Further Aspects

This article omits some aspects of liver aging already reviewed elsewhere: They prominently include the roles of stem cells [102], autophagy [103], and redox biology [104] in the context of liver regeneration as well as the contribution of aged mast cells to the progression of NAFLD, primary biliary cholangitis, and primary sclerosing cholangitis [105] in the aging liver. Readers particularly interested in these aspects will find the aforementioned topical review articles to be excellent sources of pertinent information.

Certain environmental pollutants and the exposure to electromagnetic radiation are strongly assumed to contribute to aging: First, accumulating evidence strongly indicates that environmental pollutants generally accelerate aging by promoting cellular senescence and the occurrence of age-related diseases [106]. However, we are not aware of any studies on age-dependent changes of the hepatic detoxification of discrete pollutants. Still, it is fair to assume that an aging liver that is already impaired by its reduced potential for efficient biotransformation is additionally burdened, or even overburdened, by environmental contaminants. Second, electromagnetic radiation is increasingly acknowledged to affect the regulation of aging and longevity. Specifically, UV radiation acts mutagenic, which accelerates (skin) aging, shortens life expectancy, and promotes the generation of certain malignancies. Visible light and the choice of artificial lighting impacts human health as well. This is due to the fact that, *via* the retina, neuronal circuits and circadian rhythms are modulated. As a result, the spectrum of visible light we are exposed to may influence the quality of sleep and the development of psychiatric disorders [107]. While there is currently no specific evidence of an influence of visible light on the functioning of the liver in old age, it appears plausible that the light-dependent modulation of circadian rhythms may affect certain liver functions.

Sins of Omission

In 1945, Stern and colleagues published the first original paper on 'aging' and 'liver' [108]. Therein, they elaborated on the synthesis of hippuric acid as an excretable product resulting from hepatic detoxification [109]. They found that its concentration dwindles in age and, among the elderly, particularly in patients with dementia [110]. More than 75 years later – a period during which > 19,000 papers on liver aging were published – the complete lack of follow-up studies to these findings is surprising: one should assume that, in the absence of causal therapeutic options, the possible link between hippuric acid and dementia would have been scrutinized long ago. Hence, the potential association between the age-dependent decrease in hippuric acid and the onset and/or exacerbation of dementia (and perhaps other neurodegenerative disorders as well) will be a very worth-while matter to be investigated given the increasing percentage in elderly people diagnosed with neurodegenerative disorders. A first promising approach in this direction might be based on the proven association between dementia and NAFLD as the most common chronic liver disease worldwide whose incidence increases with age [110]. Chung et al. pointed out that this association is reflected in characteristic abnormalities in the systemic levels of certain organokines, the explanatory power of which could be used to facilitate more effective approaches to the prevention or treatment of NAFLD and age-related dementia [111]. Due to the extensive body of evidence supporting an association of dementia and NAFLD, we would thus propose to investigate how the kinetics of hippuric acid relates to the age-dependent dynamic progression of NAFLD and dementia and its associated characteristic organokine changes. While Stern and colleagues had shown that the concentration of hippurate drops most prominently in aged patients suffering from dementia [108], it has been demonstrated that, conversely, elevated levels of hippuric acid correlate with improved metabolic health and hepatic steatosis [112, 113, 114, 115]. Thus, although the exact relationship between the concentration of hippuric acid and metabolic disease is not clear at this time, the level of hippurate obviously is an indicator of hepatic function and/or can reduce the degree of liver damage [115]. Perspectively, these correlations could be a starting point to aim for arriving at an improved management of those cases of dementia that develop against a background of chronic age-dependent hepatometabolic disease.

Finally, we must address an obvious deficit that affects the elderly patients entrusted to our care – and that will not spare ourselves in the future: Even though research has accumulated abundant information on age-dependent changes in the efficiency of hepatic detoxification, a complete and consistent picture on whether these processes generally deteriorate in age has not yet emerged [116]. It seems we have to admit that this shortcoming might largely be due to the fact that most clinical trials are still conducted in young individuals only. As a result, our knowledge of drug metabolism in old age has remained deplorably inadequate. Ironically, this starkly contrasts the fact that older people are prescribed significantly more medications, up to and including polypharmacy. Thus, most current clinical trial designs exhibit a glaring asymmetry that needs to be addressed urgently. Besides

providing novel and complementary data, we should not be surprised if the results of such trials will require corrections to the present insights described in this review.

Conclusions

Science undoubtedly made great strides towards an increased understanding of the biology of hepatic aging. Still, much remains to be done. Significant advances are most likely to come from studies in the fields of evolution (and their translation into clinically relevant settings), of transcriptional, posttranscriptional, and translational regulation in old age, on the distinctive immunity of the liver as well as the phenomenon of inflammaging, and last but not least, from add-on results arising from cleverly designed clinical studies in elderly subjects. Marrying the findings of such diverse disciplines may be realized by suitable consortia as well as by merging large and complementary data volumes either resulting from such joined efforts and/or from independent investigations by machine learning [117, 118, 119, 120], as combined with probabilistic modelling [121]. Such concerted efforts promise to provide much deeper insight into liver aging and thus pioneer approaches to more effective treatments for potentially life-threatening chronic liver diseases such as NAFLD and exacerbations including liver fibrosis and HCC.

Let us close with a comforting statement: Nancy Leslie Rutherford Bucher (May 4, 1913 – February 16, 2017) – principal discoverer of the age-related decline in the regenerative potential of the liver [22] and professionally highly active in the service of liver research until 2005 [122] – reached a centenarian's age [123]. Hence assuming that she was largely spared the detrimental consequences of an aging liver, one might almost like to think that she was rewarded by nature for her outstanding achievements.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Dedication

This contribution is dedicated to the memory of the one who started it all: Nancy Leslie Rutherford Bucher, M.D. (1913 – 2017)

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