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TOPIC HIGHLIGHT

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Spleen: A new role for an old player?

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Abstract

The spleen could be considered a neglected organ. To date, it has been deemed an ancillary organ in portal hypertension or an organ localization in lymphoproliferative diseases, even though it has had significant attention in infectious diseases for some time. Now, it is thought to be central in regulating the immune system, a metabolic asset and involved in endocrine function with regard to nonalcoholic fatty liver disease. The main mechanisms involved in this complex network will be critically discussed in this article.

Key words: Endocrine function; Immune system; Metabolic asset; Nonalcoholic fatty liver disease; Spleen

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SCENARIO

Due to the view that the "spleen is useless", research on this organ has lagged behind that of other organs. Since 1952, when King and Schumacker reported overwhelming post-splenectomy infection[1], there has been a growing recognition of the importance of the spleen in the human body. On the other hand, physicians often encounter spleen enlargement, i.e., splenomegaly which is almost always a consequence of other disorders. Hypersplenism is a secondary process that can arise from splenomegaly of almost any cause. In recent years, following in-depth studies of spleen organization and structure, cell function, secretion and innervations, a better understanding of the function of the spleen has been gained. It was initially accepted that the spleen not only filters blood but is an important regulation center of the body's immune-metabolic-endocrine network. However, a number of questions have arisen: Is the spleen a player or a bystander, and what are the roles of some cytokines, adipokines/growth factors and neurotransmitters in this complex mechanism? In other words, what is the contribution of the spleen to non-alcoholic fatty liver disease, is it a further expression of Metabolic Syndrome[2]?

ANATOMY

The spleen, in healthy adult humans, is approximately 11 cm (4.3 in) in length. It usually weighs 150 g (5.3 oz)

Table 1 Histology of spleen

Anatomy	Composition
Red pulp	"Sinusoids" which are filled with blood "Splenic cords" of reticular fibers "Marginal zone" bordering on white pulp
White pulp	Nodules, called Malpighian corpuscles, containing "lymphoid follicles" rich in B-lymphocytes "periarteriolar lymphoid sheaths", plenty of T-lymphocytes

Table 2 Function of the spleen

- Red pulp
  - Extramedullary hematopoiesis if required
  - Facilitating an environment wherein erythrocytes rid themselves of solid waste material
  - Blood filter for foreign material and damaged and senescent blood cells
  - Storage site for iron, erythrocytes, platelets, plasmablasts and plasma cells
  - Rapid release of antigen-specific antibodies into the circulation produced by red pulp plasma cells
  - Defense against bacteria using iron metabolism by its macrophages
- White pulp
  - T cell zone (periarterial lymphatic sheath) and B cell zone (follicles)
    - Storage site for B and T lymphocytes
    - Development of B and T lymphocytes upon antigenic challenge
    - Release of immunoglobulins upon antigenic challenge by B lymphocytes
    - Production of immune mediators involved in clearance of bacteria such as complement, opsonins, properdin and tuftsin
  - Marginal zone
    - Phagocytosis of circulating microorganisms and immune complexes by MZ macrophages
    - Development of marginal zone B lymphocytes upon TI-2 antigenic challenge
    - Blood trafficking of B and T lymphocytes
    - Release of immunoglobulins upon antigenic challenge by splenic B lymphocytes

and lies beneath the 9th to the 12th thoracic ribs. The spleen is an intraperitoneal organ with a smooth serosal surface and is attached to the retro-peritoneum by fatty ligaments that also contain its vascular supply. The splenic surfaces are described relative to their locations and are termed the diaphragmatic (phrenic) and visceral surfaces. The visceral surface is divided into an anterior or gastric ridge and a posterior or renal portion. The splenic hilum is directed antero-medially. The splenic artery and vein emerge from the splenic hilum in the form of six or more branches; the splenic artery is remarkable for its large size and tortuosity. The splenic artery is slightly superior to the vein. The spleen is part of the lymphatic system. The germinal centers are supplied by arterioles called penicilliary radicles. The spleen is derived from mesenchymal tissue (Table 1).

SPLEEN FUNCTION

Immune function (through phagocytosis, but also through T cell-mediated immunity and B cell-mediated humoral immunity) is the most important function of the spleen (Table 2). A current paradigm states that monocytes circulate freely and patrol blood vessels but differentiate irreversibly into dendritic cells (DCs) or macrophages upon tissue entry. Recently, it was shown that bona fide undifferentiated monocytes reside in the spleen and outnumber their equivalents in the circulation. The reservoir monocytes assemble in clusters in the cords of the subcapsular red pulp and are distinct from macrophages and DCs. In response to ischemic myocardial injury, splenic monocytes increase their motility, exit the spleen en masse, accumulate in injured tissue, and participate in wound healing. These observations uncover a role for the spleen as a site for storage and rapid deployment of monocytes and identify splenic monocytes as a resource that the body exploits to regulate inflammation[3]. The spleen plays a complex role in tumor immunity, which changes in the different periods of cancer[4]. The initiation of T-cell immune responses requires professional antigen-presenting cells. Emerging data point towards an important role for macrophages (Mphi) in the priming of naïve T cells. In this study we analyzed the efficiency and the mechanisms by which Mphi derived from spleen (Sp-Mphi) or bone marrow (BM-Mphi) present lymphocytic choriomeningitis virus antigens to epitope-specific T cells. It was demonstrated that because of phagosomal maturation, Sp-Mphi downregulate their ability to cross-present cell-associated, but not soluble, antigens, as they are further differentiated in culture without altering their capacity to directly present virus antigens after infection. Authors proposed that Sp-Mphi are extremely efficient at direct and cross-presentation. However, if these cells undergo further M-CSF-dependent maturation, they will adapt to be more scavenger and phagocytic and concurrently reduce their cross-presenting capacity. Accordingly, Sp-Mphi can have an important role in regulating T-cell responses through cross-presentation depending on their differentiation state[5]. The spleen is one of the centers of activity of the reticulo-endothelial system and can be considered analogous to a large lymph node, as its absence leads to a predisposition toward certain infections. Other functions of the spleen are the production of opsonins[6], properdin[7], and tuftsin[8], as well as the creation of red blood cells. While the bone marrow is the primary site of hematopoiesis in the adult, the spleen has important hematopoietic functions up until the fifth month of gestation. After birth, erythropoietic functions cease, except in some hematologic disorders. As a major lymphoid organ and a central player in the reticuloendothelial system, the spleen retains the ability to produce lymphocytes and, as such, remains a hematopoietic organ. In horses, roughly 30% of red blood cells are stored in the spleen. These red blood cells can be released when needed[9]. In humans, the spleen does not act as a reservoir for red blood cells but it can store platelets in case of an emergency. Platelets are major carriers of serotonin (5-HT) in the blood[10]. 5-HT has been reported to modulate T cell and natural killer (NK) cell proliferation. This aspect

was clearly elucidated by studies on cultures of mouse and rat spleen cells. Results showed that serotonin up-regulates mitogen-stimulated B lymphocyte proliferation through 5-HT1A receptors, thus providing an important link between this neurotransmitter and the immune system[11]. Another study using RT-PCR methods to examine the mRNA expression of 5-HT receptors in the cells of lymphoid tissues of the rat (*ex vivo* isolated spleen, thymus, and peripheral blood lymphocytes) confirmed 5-HT receptors (5-HT1B, 5-HT1F, 5-HT2A, 5-HT2B, 5-HT6, and 5-HT7) in mitogen-stimulated spleen cells. In contrast, 5-HT1A, 5-HT1D, 5-HT2C, 5-HT4, 5-HT5A, and 5-HT5B mRNAs were not detected in any of the examined cell populations[12]. The role of platelets and serotonin was recently highlighted as novel contributors in the mechanisms of liver regeneration after partial hepatectomy[13]. Furthermore, platelets are attracted to the liver following systemic inflammatory stimuli[14].

ASSESSMENT OF SPLEEN FUNCTION

Patients with impaired splenic function are difficult to identify[15]. IgM memory B cells are a potential parameter for assessing splenic function[16]; however, more studies are necessary for its validation. The detection of Howell-Jolly bodies does not reflect splenic function accurately[17], whereas determining the percentage of pitted erythrocytes is a well-evaluated method and seems a good first-line investigation for assessing splenic function[18]. When assessing spleen function, (99m)Tc-labeled, heat-altered, autologous erythrocyte scintigraphy with multimodality single photon emission computed tomography (CT)-technology is the best approach, as all facets of splenic function are evaluated[19].

THE BLOOD-SPLEEN-BARRIER

The blood-spleen-barrier (BSB) is a barrier composed of macrophages and endothelial cells of the marginal sinus. Their basement membrane is composed of reticular tissue (reticular cells and reticular fibers) and collagen fibers. It can regulate splenic filtration and its intrasplenic consequences including blood flow, cell homing and migration, hematopoietic and immune responses, and clearance of infectious organisms. Here, the cells of the barrier can trap circulating infectious organisms and monocytes on their cell surfaces, clearing them from the blood and providing a selective environment for monocyte differentiation into macrophages and subsequent phagocytosis of the microorganisms. The interactions between the circulating lymphocytes and the macrophages may regulate the entry of lymphocytes into the white pulp. Thus, the functions of the BSB are to filter antigens, to keep the microenvironment of the white pulp stable, and to present antigen information to white pulp through the effects of the mechanical barrier, which depends on the connection between cells and the phagocytosis of macrophages. Compared to other biological barriers in the human body, such as the blood-brain barrier and the blood-thymus barrier, the structure of the BSB is relatively loose without the tight junction between cells; however, the BSB has more constituents and ability to stop and phagocytize more xenobiotic materials than other barriers[20,21]. As compared to the normal spleen, the density of macrophages in the portal hypertension (PH) spleen was decreased, but the macrophages were mainly located in the marginal zone and distributed around the splenic corpuscle, with many villi and pseudopodium-like protrusions on the cell surface. The accrementition of collagen fibers was obvious around the splenic corpuscle and central artery. The increased reticulate fibers encircled the splenic corpuscle with more connection between the fibers. The vascular endothelial cells were in diffused distribution, without any regionality in PH spleen, but the vessel with enlarged lumina increased in red pulp[22].

THE OLD PLAYER

Except for malaria and genetic metabolic diseases (e.g., Gaucher disease), splenic enlargement can be caused by diseases such as PH, lymphoma and leukemia. PH is considered the most common cause of splenomegaly in Western countries. Previous findings showed that splenomegaly is secondary to PH with associated liver cirrhosis. In fact, the increase in the width of the celiac axis in cirrhotic patients with PH was closely related to the increased width of the splenic artery which in turn was related to enlargement of the spleen, and increased blood flow through the spleen. The increased size of the spleen is due partly to venous engorgement and partly to reticulo-enthothelial cell hyperplasia, and is accompanied by an increased total blood supply, although flow per 100 g tissue is often reduced. An increase in blood flow can not occur without dilatation of the entire splenic arterial tree (Pouseille's law) and in keeping with this are the studies of[23], using injected spleen casts, which showed an increased number of peripheral arterioles of 100 mm diameter. In addition to local factors, circulating vasodilator substances may sometimes have an additional effect. The cardiac output is often raised, the blood flow through skin and muscle is also increased, and there is evidence of an increased number of small peripheral arterioles in the lungs. All these findings show that a generalized vasodilatation may occur in some patients with cirrhosis. Increasing tortuosity of peripheral vessels is a well-known accompaniment of aging, but in cirrhotic patients there was no relationship between tortuosity and either age or length of history. The increased length and tortuosity of the splenic artery is probably a secondary effect of arterial dilatation, although there was no direct relationship either to total splenic blood flow or size of the spleen. This was particularly striking in patients with tropical splenomegaly who had enormously enlarged spleens, increased blood flows, but splenic arteries of normal length[24]. Currently, there is controversy on the immune function of enlarged spleen in patients with PH and hypersplenism. As compared to the normal spleen, the density of macrophages in the PH spleen was decreased, but the macrophages were mainly located in the marginal zone and distrib-

uted around the splenic corpuscle, with many villi and pseudopodium-like protrusions on the cell surface. The "accrementition", i.e., growth by addition of similar collagen fibers, was obvious around the splenic corpuscle and central artery. The increased reticulate fibers encircled the splenic corpuscle with more connection between the fibers. The vascular endothelial cells were in diffused distribution, without any regionality in PH spleen, but the vessel with enlarged lumina increased in red pulp. Those morphological changes of the BSB may be one of the pathological fundaments for the abnormality of immune function and the increased destruction of blood cells located in the spleens of patients with PH[22].

Lymphoma is the commonest malignant tumor of the spleen. Although a number of lymphomas and leukemias can involve the spleen and may present clinically with splenomegaly, only the B cell disorders SMZL and hepato-splenic γ/δ T cell lymphoma can be considered true primary splenic lymphomas[25]. It is important to detect splenic involvement because it can alter the management and for this reason Gadolinium-enhanced sequences are sensitive.

LIVER CIRRHOSIS, SPONTANEOUS SPLENORENAL SHUNT AND HYPERSPLENISM

Although significant advances are expected to be made in the assessment of PH-related complications, the prognostic role of spleno-renal shunts (SRS) has not been fully explored so far. Clarifying this aspect could help tackle the life-threatening events occurring in patients suffering from liver cirrhosis. A recent study on SRS[26] focused on the role of the spleen and showed a strict link between spleen size and the presence of SRS and the development of hepatocarcinoma.

An up-to-date study evaluated the effect of liver transplantation on spleen size, spontaneous SRS function, and platelet counts in patients with hypersplenism in 462 adult patients who underwent orthotopic liver transplantations (OLTX). Of these patients, CT or magnetic resonance imaging information was reviewed retrospectively in 55 patients. Volume measurements of the spleen and liver, spleen/liver volume ratio (S/L ratio), presence and size of SRS, and platelet counts were evaluated before and after OLTX. Spleen size and SRS size were significantly smaller after OLTX. However, patients with postoperative S/L ratio > 0.35 tended to have lower platelet counts after OLTX[27].

THE NOVEL PLAYER

Nonalcoholic fatty liver disease (NAFLD), the most common cause of steatosis, is associated with obesity, mainly visceral and insulin resistance. In the presence of more severe risk factors (major obesity, diabetes mellitus, metabolic syndrome, MS), simple hepatic steatosis or fatty liver (FL) may be complicated by liver inflammation (nonalcoholic steatohepatitis or NASH). NASH can lead to perisinusoidal fibrosis and cirrhosis. Fat-laden hepatocytes are swollen, and in steatohepatitis, further swelling occurs due to hydropic change (ballooning) of hepatocytes to cause sinusoidal distortion, as visualized by *in vivo* microscopy, reducing intrasinusoidal volume and microvascular blood flow. Involvement of other cell types (sinusoidal endothelial cells, Kupffer cells, stellate cells) and recruitment of inflammatory cells and platelets lead to dysregulation of microvascular blood flow. In animal models, the net effect of such changes is a marked reduction of sinusoidal space (approximately 50% of control), and a decrease in the number of normally perfused sinusoids. Such microvascular damage could accentuate further liver injury and disease progression in NASH. Hepatic steatosis is also exquisitely sensitive to ischemia-reperfusion injury, at least partly due to the propensity of unsaturated fatty acids to undergo lipid peroxidation in the face of reactive oxygen species. This has important clinical consequences, particularly limiting the use of fatty donor livers for transplantation[28]. NASH is a progressive liver disease characterized by Kupffer cell dysfunction which contributes to its pathogenesis. It is noteworthy that the reticular-endothelial system also plays a key role in the spleen. Colloid scintigraphy is a good method of reflecting Kupffer cell activity. A study on 22 patients with biopsy-proven NASH who underwent colloid liver scintigraphy, after intravenous injection of 185 MBq Tc tin colloid, showed that liver right/left lobe ratio was altered in all of these patients. Colloid shift to the spleen was observed in 55% of patients as well as prolonged blood pool clearance time[29].

The first group of researchers[30] who aimed to determine if there was an association between NAFLD and spleen enlargement, measured spleen volume using CT. The values were compared with the patient's demographic data, the liver-to-spleen (L/S) ratio of CT Hounsfield unit measurements, and the results of liver function tests. Diagnosis of fatty liver was made if the L/S ratio was less than 1.0. The mean spleen volume was 73.0 ± 24.4 cm^3 (range, 21.1-106.1) in normal subjects and 141.2 ± 54.1 cm^3 (range, 44.1-267.3) in patients with fatty liver ($P < 0.0001$). Multivariate linear regression analysis identified that only the L/S ratio ($P < 0.0001$) and age ($P < 0.01$) were significantly correlated to spleen volume. Using forward selection stepwise regression, the L/S ratio entered first (β = -0.634) and age second (β = - 0.293).

Obesity and insulin resistance are strongly associated with systemic markers of inflammation. Focusing on this aspect, authors have attempted to find a noninvasive method that could likely assess the presence of NASH and help to decide liver biopsy performance. Using histology as a gold standard to diagnose NAFLD, 43 patients with NASH and 40 with fatty liver were consecutively studied, their data were compared with those of 48 healthy control participants. The outcomes evaluated were ultrasonographic spleen longitudinal diameter coupled with the splenic artery resistive index, serum interleukin (IL)-6 and vascular endothelial growth factor

concentrations. The NASH group had higher spleen longitudinal diameter values ($P = 0.0001$) as well as significantly higher IL-6 and vascular endothelial growth factor concentrations than the other groups ($P = 0.0001$). The optimal cut-off value for spleen longitudinal diameter that best discriminated NASH from fatty liver patients was 116 mm (specificity 95% and sensitivity 88%); the sensitivity and specificity of this parameter was better than both IL-6 and vascular endothelial growth factor in the same setting (area under the receiver operating characteristic curve 0.920 *vs* 0.817 and 0.678, respectively). Splenic artery resistive index was similar between patients with NASH and those with fatty liver, but differed when compared with controls ($P = 0.0001$). IL-6 was highly specific in confirming the absence of NASH at normal values. In that series of patients, normal values of spleen longitudinal diameter and IL-6 were strongly associated with fatty liver[31]. Further confirmation of these findings comes from another study which highlighted that spleen enlargement may be a distinct feature of NASH, especially early-stage NASH[32].

A subsequent study[33] showed that spleen enlargement was found at significant levels (38%) in obese patients as determined by Cavalieri stereologic volume calculation, an unbiased stereological method. Finally, recent results clearly indicated that high fat diet caused splenomegaly *via* sinusoidal dilatation and intracellular or intercellular deposits in obese female rats[34]. Although in patients with NAFLD, liver biopsy remains the only reliable method to differentiate simple steatosis from NASH, the objective of the study was to evaluate the efficacy of non-invasive (99m)Tc-phytate scintigraphy in the diagnosis of NASH. Thirty-seven patients with suspected NAFLD at the time of liver biopsy also underwent (99m)Tc-phytate scintigraphy. Signal intensities of regions of interest in the liver and spleen were measured. The same authors also examined scintigraphic features in a nutritional model of NASH in rats fed a methionine- and choline-deficient (MCD) diet. The liver/spleen uptake ratio determined by scintigraphy was significantly decreased in patients with NASH in comparison with patients with simple steatosis. The liver/spleen ratio was an independent predictor distinguishing NASH from simple steatosis. The decrease was observed for all stages of NASH, including the early stage (stages 1 and 0). In animal studies, the liver/spleen uptake ratio was significantly decreased in rats after 8 wk of a MCD diet in comparison with control diet-fed rats. These authors concluded that non-invasive (99m)Tc-phytate scintigraphy is a reliable tool to differentiate NASH from simple steatosis[35]. The frequency of ischemic heart disease observed after splenectomy for trauma and the low cholesterol levels found in patients with hypersplenism are observations that suggest a possible role for the spleen in lipid metabolism and in the etiology of atherosclerosis[36,37]. Previous studies showed that obese subjects are more susceptible to cardiovascular disease, hypertension, cerebrovascular disease, and diabetes mellitus than are non-obese subjects. They have a higher incidence of infection and some types of cancer, suggesting impaired immune function. In humans, only a few studies have directly compared specific immune responses in obese and non-obese subjects. It is known that obesity induces decreases in both T lymphocyte response to concanavalin A and B lymphocyte response to pokeweed mitogen[38]. In addition, a negative correlation between percentage body fat and natural killer cell activity was found in both elderly women[39], and adult men[40]. Elderly people (> 60 years of age) are also at risk of an increased incidence of infection. Their peripheral blood lymphocytes show an impaired proliferative capacity and a decreased reactivity to mitogens[41]. Researchers found and reported that obesity suppresses lymphocyte functions, natural killer cell activity, and lymphocyte mitogenesis in men and women > 60 years of age[42]. This suggests that obesity is a risk factor for deteriorating cellular immune functions. However, the mechanism by which obesity decreases cellular immune functions remains to be elucidated. Expression of glucose transporter 1 (GLUT-1), analyzed by Western blot analysis, was lower in the splenic lymphocytes of obese compared with lean Zucker rats. In obese subjects it is associated with the decreased uptake of glucose into immune cells, which in turn is associated with the decreased expression of GLUT-1. This suggests that decreased proliferation of splenic lymphocytes in obese Zucker rats is associated with the impairment of glucose uptake, which is due to the decreased expression of GLUT-1[43]. An up-to-date study assessed the magnitude of antigen-specific immunity in a murine model of NAFLD. Because antigen-specific immunity was diminished in NAFLD mice, the underlying mechanisms were evaluated through analysis of the functions of antigen-presenting DC and other immunocytes. For 12 wk, NAFLD mice received a high-fat and high-calorie diet. NAFLD mice and control mice were immunized with hepatitis B vaccine containing hepatitis B surface antigen (HBsAg) and hepatitis B core antigen (HBcAg). Antibody to HBsAg (anti-HBs), HBsAg and HBcAg-specific cellular immune response and functions of whole spleen cells, T lymphocytes, B lymphocytes and spleen DCs of NAFLD and control mice were assessed *in vitro*. Levels of anti-HBs and the magnitude of proliferation of HBsAg and HBcAg-specific lymphocytes were significantly lower in NAFLD mice than control mice. The spleen cells of NAFLD mice produced significantly higher levels of inflammatory cytokines and exhibited significantly increased T cell proliferation compared with control mice. However, the antigen processing and presenting capacities of spleen DCs were significantly decreased in NAFLD mice compared with control mice. Palmitic acid, a saturated fatty acid, caused diminished antigen processing and presenting capacity of murine DCs[44]. Liver fat represents a balance between input, secretion, and oxidation of fatty acids. As humans spend the majority of a 24-h period in a postprandial state, dietary fatty acids make an important contribution to liver fat metabolism. Oxidation of dietary fatty acids, hepatic desaturation and elongation of palmitic acid occurs to a greater extent in abdominally obese men.

NEUROTRANSMITTER, HORMONES, VITAMIN D AND THE SPLEEN

Increasing evidence has placed hormones and neurotransmitters among potent immunomodulators, in both health and disease. 5-HT functions as a neurotransmitter in the nervous systems of simple as well as complex animals. 5-HT diffuses to serotonin-sensitive neurons, which control the animal's perception of nutrient availability. This system has been partially conserved during the 700 million years of evolution which separates *C. elegans,* a transparent nematode, from humans. When humans smell food, dopamine is released to increase the appetite. However, unlike that in worms, serotonin does not increase anticipatory behaviour in humans; instead the serotonin released while consuming activates 5-HT_{2C} receptors on dopamine-producing cells. This halts their dopamine release, and thereby serotonin decreases appetite. Drugs which block 5-HT_{2C} receptors make the body unable to shut off appetite, and are associated with increased weight gain[45], especially in people who have a low number of receptors[46]. The expression of 5-HT_{2C} receptors in the hippocampus follows a diurnal rhythm, just as the 5-HT release in the ventromedial nucleus, which is characterized by a peak in the morning when the motivation to eat is strongest[47]. In humans, serotonin levels are affected by diet. An increase in the ratio of tryptophan to phenylalanine and leucine will increase serotonin levels. Fruits with a good ratio include dates, papaya and banana. Foods with a lower ratio inhibit the production of serotonin. Research also suggests that eating a diet rich in carbohydrates and low in protein will increase serotonin by secreting insulin, which helps in amino acid competition[48]. However, increasing insulin for a long period may trigger the onset of insulin resistance, obesity, type 2 diabetes, and lower 5-HT levels. Researchers showed that expression of 5-HT(2A) receptors was up-regulated in hypertrophic 3T3-L1 adipocytes, which exhibited decreased expression of adiponectin and increased expression of PAI-1. 5-HT(2A) receptor antagonists and suppression of 5-HT(2A) receptor gene expression enhanced adiponectin expression. Activation of Gq (the G protein-coupled receptor is activated by an external signal in the form of a ligand or other signal mediator) negatively regulated adiponectin expression, and inhibition of mitogen-activated protein kinase reversed the Gq-induced effect. Moreover, the 5-HT(2A) receptor blockade reduced PAI-1 expression[49]. As food intake and energy balance are among the functions regulated by 5-HT in the brain, it would be interesting to discover its link with some adipokines. Recent studies have shown an interaction between the serotonergic system and leptin, a protein released from adipose tissue that inhibits feeding behavior and increases fuel expenditure. An up-to-date study found low brain serotonin immunoreactivity in all animals with high neuronal leptin accumulation in the raphe nucleus, independently of their age. In contrast, high brain serotonin immunoreactivity was accompanied by a low neuronal accumulation of leptin. These findings indicate that serotonin regulates leptin uptake by neuronal cell bodies of the dorsal raphe and hypothalamus, suggesting that at least part of the effects of serotonin may be mediated by the regulation of neuronal trafficking in the brain[50]. 5-HT promotes the release of growth hormone (GH) by a hypothalamic site of action[51]. Exogenous GH enhances thymic microenvironmental cell-derived secretory products such as cytokines and thymic hormones. Moreover, GH increases thymic epithelial cell (TEC) proliferation *in vitro*, and exhibits a synergistic effect with anti-CD3 in stimulating thymocyte proliferation, which is in keeping with data showing that transgenic mice overexpressing GH or GH-releasing hormone exhibit overgrowth of the thymus. GH also influences thymocyte traffic: it increases human T-cell progenitor engraftment into the thymus; augments TEC/thymocyte adhesion and the traffic of thymocytes in the lymphoepithelial complexes, the thymic nurse cells; modulate *in vivo* the homing of recent thymic emigrants, enhancing the number of fluorescein isothiocyanate positive cells in the lymph nodes and diminishing them in the spleen. In keeping with the effects of GH on thymic cells, is the detection of GH receptors in both TEC and thymocytes. Insulin-like growth factor (IGF)-1 is a potent hormone that stimulates growth and differentiation and inhibits apoptosis in numerous tissues. Preliminary evidence suggests that IGF-1 exerts differentiating, mitogenic and restoring activities in the immune system, however, the sites of synthesis of local IGF-1 are unknown. Identification of these sites would allow the functional role of local IGF-1 to be clarified. The presence of IGF-1 in non-immune cells suggests that it acts as a trophic factor, while its occurrence in subtypes of lymphocytes or antigen-presenting cells indicates paracrine/autocrine direct regulatory involvement of IGF-1 in the human immune response. Additionally, data indicate that IGF-1 is involved in several effects of GH in the thymus, including the modulation of thymulin secretion, TEC proliferation as well as thymocyte/TEC adhesion. This is in accordance with the demonstration of IGF-1 production and expression of IGF-1 by TEC and thymocytes. Also, it should be seen as an intrathymic circuitry, involving not only IGF-1, but also GH itself, as intrathymic GH expression is seen both in TEC and in thymocytes, and that thymocyte-derived GH could enhance thymocyte proliferation[52]. With regard to the implication of the IGF family in immune physiology and development, a recent study has focused on type 1 IGF receptor, a transmembrane tyrosine kinase homologous to the insulin receptor that mediates most of the biological effects of IGF-1 and IGF-2. Normal development and *ex vivo* activation of T and B cells are observed in chimeric *Rag2*-deficient C57BL/6 mice reconstituted with fetal liver cells from *Igf1r*$^{-/-}$ mice. However, this model revealed an unexpected decrease in the T-independent B cell response which is important in bacterial defense mechanisms[53]. The major role of IGF-2 is as a growth promoting hormone during gestation. To date, very few studies have investigated the function of IGF-2 in immune development and physiology.

This growth factor is the dominant peptide of the insulin family expressed in the thymus epithelium of different species. Thymic IGF-2 influences thymic development and T cell differentiation as evidenced by the analysis of IGF-2 transgenic dwarf mice, which develop thymic hyperplasia with an increased number of thymocytes (and $CD4^+$ T lymphocytes in particular). This increase in T cells is also observed in the spleen compartment of IGF-2 transgenic mice, but there is no significant effect on B cell development[54]. There is further evidence that IGF-2 may intervene in the control of T cell differentiation[55]. A recent study investigated the location of IGF-1 messenger RNA and protein on archival human lymph node samples by *in situ* hybridization, immunohistochemistry and double immunofluorescence staining using an IGF-1 probe and antisera specific for human IGF-1 and CD3 (T lymphocytes), CD20 (B lymphocytes), CD68 (macrophages), CD21 (follicular DCs), S100 (interdigitating DCs) and podoplanin (fibroblastic reticular cells). Numerous cells within the B- and T-cell compartments expressed the IGF-1 gene, and the majority of these cells were identified as macrophages. Solitary follicular DCs exhibited IGF-1. A few T lymphocytes, and no B lymphocytes, contained IGF-1 immunoreactive material. Furthermore, IGF-1 immunoreactive cells outside the follicles that did not react with CD3, CD20, S100 or podoplanin markers were identified as high-endothelial venule cells[56]. GH was used to counteract the catabolic metabolism in critically ill patients until it was demonstrated that administration of GH was associated with increased morbidity due to uncontrolled infections and sepsis[57]. The immunomodulatory effect of GH and its main mediator IGF-1 during systemic inflammation remain to be established. Authors investigated the effect of GH and IGF-1 on cellular immune functions in a murine model of sepsis and found that GH did not affect cellular immune functions or the survival rate in that model. In contrast, IGF-1 improved splenocyte proliferation and cytokine release independently of GH but did not affect the determined clinical parameters of septic mice[57]. Aging is under the control of a small number of regulatory genes. Mice genetically selected for high immune responses, in most cases, exhibit a longer life span and lower lymphoma incidence than do mice selected for low responses. The link between immunity and aging is further evidenced by the age-related alterations in the immune system, mostly of the T-cell population, in terms of replacement of virgin by memory cells, accumulation of cells with signal transduction defects, and changes in the profile of Th1 and Th2 type cytokines[58]. Also, B cells exhibit intrinsic defects, and NK cell activity is profoundly depressed by aging. *In vitro* experiments indicate that the production of IL-2, interferon (IFN)-γ, and IL-4 by mouse spleen cells changes with aging and may be upregulated by recombinant cytokines. These findings suggest possible cytokine interventions to prevent or treat age-related immune disorders, as they may affect the duration and the biological quality of life[59]. Excessive alcohol consumption continues to be a major public health problem, particularly in the adolescent and young adult populations. Generally, such behavior tends to be confined to the weekends, resulting in frequent binge drinking. Various authorities have emphasized the strict link between mechanisms inducing alcoholic and nonalcoholic liver diseases, thus it could be of interest to ask questions about alcohol toxicity, such as: is there a link between alcohol abuse and impaired immune system and what is the link? A study in peri-pubertal male rats compared the effect of the discontinuous feeding of a liquid diet containing a moderate amount of ethanol (6.2% wt/vol) to that of continuous ethanol administration or a control diet, taking as end points the 24-h variations in plasma prolactin levels and mitogenic responses and lymphocyte subset populations in the spleen. Animals received the ethanol liquid diet starting on day 35 of life, the diet being similar to that given to controls except that maltose was iso-calorically replaced by ethanol. Ethanol provided 36% of the total caloric content. Each week, the discontinuous ethanol group received the ethanol diet for 3 d and the control liquid diet for the remaining 4 d. After 4 wk the rats were killed. A significant decrease in splenic cell response to concanavalin A, and of splenic cell response to lipopolysaccharide was found in rats under the discontinuous ethanol regime, when compared with control- or ethanol-chronic rats. Under discontinuous ethanol feeding, mean values of splenic CD8(+) and CD4(+)-CD8(+) cells decreased, whereas splenic T cells, and splenic B cells were augmented. In rats chronically fed with ethanol, splenic mean levels of CD8(+) and CD4(+)-CD8(+) cells were augmented. Both modalities of ethanol administration disrupted the 24 h variation in immune function seen in controls. Mean plasma prolactin levels increased by 3.6-fold and 8.5-fold in rats chronically or discontinuously fed with alcohol, respectively. These results supported the view that the discontinuous drinking of a moderate amount of ethanol can be more harmful for the immune system than continuous ethanol intake, presumably by inducing greater stress as indicated by the augmented plasma prolactin levels observed[60]. Numerous studies have focused their attention on the role played by vitamin D in obesity, MS and NAFLD. The hormonal form of vitamin D, 1,25-dihydroxyvitamin D3, is well known for its immunosuppressive, anti-proliferative and pro-apoptotic activities. In a recent work, authors studied the effect of 1,25-dihydroxyvitamin D3 on *Toxoplasma gondii*-infected mice. They observed that 1,25-dihydroxyvitamin D3 reduces the survival rate of infected mice by up to 37% at day 10 post-infection compared to untreated infected mice ($P < 0.0001$). IFN-γ and IL-12p40 levels were significantly reduced by 1,25-dihydroxyvitamin D3 in infected mice sera indicating an inhibition of Th-1-type cytokines. CD4+ T lymphocyte and splenocyte counts were also reduced following 1,25-dihydroxyvitamin D3 treatment and a marked induction of apoptosis, accompanied by down-regulation of the anti-apoptotic proteins Bcl-2 and Bcl-X(L), was observed. The above results indicate that 1,25-dihydroxyvitamin D3 induces splenocyte apoptosis and enhances host suscepti-

bility to toxoplasmosis[61]. Bone components participate in the regulation of hematopoietic stem cells (HSC) in the adult mammal. Vitamin D regulates bone mineralization and is associated with pleiotropic effects in many cell types including putative roles in hematopoietic differentiation. Researchers reported that deletion of the vitamin D receptor (VDR) in hematopoietic cells did not result in cell autonomous perturbation of HSC or progenitor function. However, deletion of VDR in the microenvironment resulted in a marked accumulation of HSC in the spleen that could be reversed by dietary calcium supplementation. These data suggest that VDR participates in restricting splenic hematopoiesis through maintenance of bone calcium homeostasis and are consistent with the concept that calcium regulation through VDR is a central participant in localizing adult hematopoiesis preferentially to bone marrow[62].

CONCLUSION

This special organ should be taken into account when interpreting the mechanisms of NAFLD and in its diagnosis, mainly when dealing with the more severe form, i.e., NASH, although recent research has challenged the benignity of FL[63].

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