Antibodies as Biomarkers: Effect of Microcystin Exposure

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Abstract

Microcystins are toxins produced by cyanobacteria of the genus *Microcystis*. When cyanobacteria proliferate in fresh or brackish waters, microcystins become a risk for public health, particularly in water bodies used as a drinking supply and for recreation. *Microcystis* spp. and microcystins (MCs) are globally distributed, and under climate change conditions, this phenomenon is expected to increase, and so is the health risk. MCs are mainly hepatotoxic; the intoxication is difficult to diagnose because its symptoms are nonspecific and easily confused with those of other diseases. Although there is vast evidence of the health problems associated with this exposure, there are no simple and specific biomarkers available to help evaluate the epidemiological impact and the extent of human exposure or even to diagnose the intoxication. This chapter reviews the toxicity mechanisms of MCs and the biochemical parameters altered when exposed to MCs. The value of specific antibodies as potential biomarkers is also discussed.

Keywords

Algal blooms · Cyanobacteria · *Microcystis* spp. · Cyanotoxins · Microcystins · Microcystin-LR · Intoxication · Antibodies · Exposure biomarker · Chronic exposure · Acute exposure · Hepatotoxicity

Abbreviations

Abs	Antibodies
ADDA	3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid
ADME	Absorption, distribution, metabolism, and excretion
ALP	Alkaline phosphatase
ALT	Alanine transaminase
AST	Aspartate transaminase
ATCC	American type culture collection
CaMKII	Calcium-calmodulin-dependent protein kinase II
ERK	Extracellular signal-regulated kinases
GGT	Gamma glutamyltransferase
GRP94	Glucose-regulated protein 94
GSH	Reduced glutathione
HL7702	Normal human hepatic cell line
Igs	Immunoglobulins
JNK/SAPK	Mammalian stress-activated protein kinase
LDH	Lactate dehydrogenase
MAPK	Mitogen-activated protein kinases
MC	Microcystin
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MC-LR	Microcystin-LR

MCs	Microcystins
MCV	Mean corpuscular volume
OATPs	Organic anion transporting polypeptides
p38	Mammalian stress-activated protein kinase
PLT	Platelets
PP1	Protein phosphatase 1
PP2A	Protein phosphatase 2A
RBC	Red blood cells
SOD	Superoxide dismutase
WBC	White blood cells

Introduction: Cyanobacteria, Their Toxins, and Health Risk

In recent decades, the excessive growth of cyanobacteria in different water bodies (rivers, lakes, reservoirs, and estuaries) has become an increasingly relevant sanitary problem (Carmichael and Boyer 2016; Cheung et al. 2013; Reichwaldt and Ghadouani 2012), which is expected to become even worse during the next years as a consequence of a changing climate and the environmental crisis (Michalak et al. 2013; Paerl and Huisman 2008). Cyanobacteria are also known as blue-green algae, and they are part of the phytoplankton community in the aquatic ecosystems. These bacteria are ancient microscopic organisms that can survive in fresh waters, in brackish waters, and even in soil – either on the surface or beyond it – according to their strain. When they reproduce excessively in aquatic environments, they form bodies of floating cyanobacteria, a phenomenon known as cyanobacterial blooms. This phenomenon usually results from a high input of nutrients like phosphorus (P) and nitrogen (N) due to human activity – a process commonly named eutrophication – and to a temperature higher than 15 °C (Gilbert 2017). For these reasons, blooms are more frequent during the summer. When they occur, organic matter from phytoplankton biomass floats in the water surface conferring a specific odor and an intense blue-green color (WHO 2015).

Some cyanobacterial strains, though not all of them, have the ability to produce toxins. These toxins can be classified based on their chemical structure, their toxicity effect, and their origin. They are generally known as cyanotoxins. The most frequent are anatoxins, nodularins, and microcystins, which were originally named after the cyanobacteria genus that was first observed to produce them (*Anabaena*, *Nodularia*, *Microcystis*, respectively). Microcystins (MCs) – one of the most abundant in water bodies – share among them (and with nodularins) a non-proteogenic aminoacidic group named ADDA (3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid) (Carmichael 1992). Up to date, more than 250 congeners of microcystins have been characterized, with molecular weights that range between 800 and 1100 Da (Fastner and Humpage 2021).

The ability of cyanobacteria to produce toxins makes blooms a general health problem, and this fact has been known for more than 200 years (Francis 1878). Ample evidence indicates that there is a proven risk when contaminated waters are

consumed and also during recreational activities that involve contact with it (Backer et al. 2010). This risk has been documented for numerous animal species besides human beings, like sheep, cattle, horses, pigs, dogs, fish, rodents, amphibians, ducks, bats, flamingoes, zebras, and rhinoceros (Valerio et al. 2010). Their relevance for human health is so well known that the WHO considered this as an emerging issue in water (WHO 2003) and has provided water quality guidelines for testing cyanotoxins in drinking and recreational waters (WHO 2020). Nevertheless, there is no established method yet for diagnosing intoxication with cyanotoxins (Su et al. 2020).

Toxic algal blooms are a worldwide problem, and South America is not exempt from them; yet information concerning algal blooms in this region is still scarce (Svirčev et al. 2019). In Argentina, an increasing number of episodes have been documented since 1990, probably in association with changes in human activities, like urbanization, the introduction of new agricultural practices, and the discharge of organic matter into water bodies without the corresponding prior treatment. Although the negative consequences for health and for the environment are evident, regulation in Argentina is far behind other countries in the region, like Uruguay and Brazil, which have, for example, incorporated cyanotoxin surveillance in recreational water and water for consumption as a routine practice (Aguilera et al. 2018). Brazil has a long history of algal blooms with cyanobacteria. According to some authors, the frequency of these blooms justifies the implementation of surveillance strategies on water reservoirs, considering their high toxicity for humans and mammals in general (Bittencourt-Oliveira et al. 2014). The problem also affects other countries from the Southern Cone, like Uruguay, which revised the incidence of cyanobacterial blooms and contamination of water bodies in research papers from 2015 (Bonilla et al. 2015) and 2020 (Juanena et al. 2020), and Chile (Campos et al. 2005), which has studied algal blooms in continental water bodies as a part of an approach to a problem that has been affecting them with increasing concern over the last three decades.

According to reports from many parts of the globe, it is commonly accepted that intoxication with cyanotoxins might occur through skin contact (dermal exposure route, when contaminated waters come into contact with skin, e.g., during recreational activities), through the respiratory tract (inhalation route, when water enters via inhalation during recreational activities), through the digestive tract (oral route, when contaminated water or food is ingested), and other more rare situations like during dialysis treatment (intravenous route) (Poste et al. 2011; USEPA 2015). Depending on the exposure route, the exposure time, the magnitude of the bloom, and the ability of cyanobacteria to produce toxins, the result can be an acute or a chronic clinical condition. In some extreme cases, the intoxication can be lethal.

Although not frequent, there are several reports concerning clinical symptoms due to acute exposure to microcystins. An excellent revision regarding this subject is the one published by Wood (2016), in which the authors suggest that the lethal dose (LD_{50}) of MC-LR is about ten times lower than that of strychnine and 200 times lower than sodium cyanide. Besides, the authors point out that the relation between toxicity and dose has a sharp curve with an abrupt slope; thus, when toxic effects

appear, the dose is already close to being lethal. In that revision, acute cases are described from 1930 until the present day, including a wide spectrum of circumstances, ranging from recreational exposure to contamination during medical procedures and occupational contact (Wood 2016). In general, it is considered that an acute clinical condition can be mild, moderate, or critical, and it is characterized by gastrointestinal symptoms (nausea, vomiting, fever, headache), a respiratory syndrome (asthma, pneumonia, cold symptoms), allergic reactions (rash, dermatitis, skin peeling), and hepatic toxicity, which can be followed by multiple organic failure and death (Pérez et al. 2008; Testai et al. 2016).

On the other hand, the available evidence regarding chronic exposure to toxic algal blooms and its impact on health is overwhelming. Moreover, the number of chronic cases may be underestimated due to both a deficient sanitary control of water quality and the poor knowledge medical practitioners have of this type of intoxication. This underestimation may be exacerbated by the many factors that remain unknown on this subject and by the lack of clear indicators to be used in the diagnostic algorithm of toxicity by cyanotoxins. Concerning these issues, Funari and Testai (2008) indicated that the sanitary risks associated with chronic exposure to cyanotoxins deserve to be considered critical, far beyond the risks associated with acute exposure. In agreement with this concern, the American Academy of Pediatrics (AAP) published a warning document in 2015 regarding the risks associated with the chronic consumption of water contaminated with cyanobacteria, particularly for infants and children (Etzel 2015).

In this review, we have focused on the molecular mechanisms of MCs' toxicity, the effects of chronic exposure to microcystins, and the biochemical indicators that have been used so far to identify intoxication. As the current status of knowledge on specific and easily measurable biochemical indicators used as biomarkers is still insufficient, we here introduce some new insights into the potential use of antibodies as specific and straightforward biomarkers of exposure and for their potential use in routine analyses.

Microcystins: Structure and Toxicity Mechanisms

Chemical Structure

As we have mentioned before, microcystins are the most studied toxins produced by cyanobacteria and the most frequently found in algal blooms. These molecules are cyclic heptapeptides composed of five non-proteogenic amino acids and two additional L-amino acids, which vary from molecule to molecule and are responsible for more than 250 microcystin variants. Nevertheless, the most frequent microcystin is MC-LR, which contains leucine and arginine. Figure 1 shows a general structure for MCs (Fastener and Humpage 2021).

According to the literature, residues 5 and 6 in Fig. 1 (ADDA group and D-glutamic acid) are responsible for the ability of MCs to bind and inhibit phosphatases, as described in the following section, although it has been stated that residues

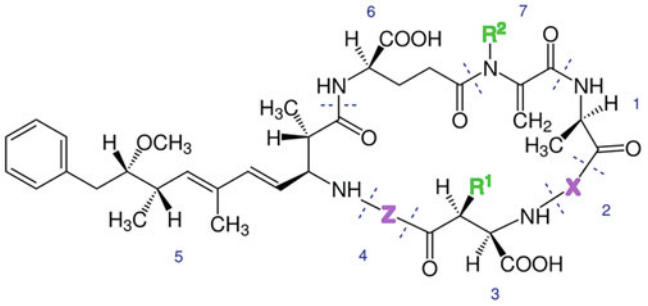

Fig. 1 General structure of microcystins. In the structure, X and Z (violet, positions 2 and 4, respectively) are the variable L-amino acids (in MC-LR, X stands for L-leucine and Z stands for L-arginine); R1 and R2 can be either H or CH3 (green); the rest of the residues are (1) D-alanine, (3) D-erythro-β-methylaspartic acid, (5) ADDA group, (6) D-glutamic acid, (7) N-methyl-dehydroalanine

X and Z confer different hydrophilicity profile to the molecule, and this has been linked to toxicity levels in animal models (McLellan and Manderville 2017; Vesterkvist et al. 2012).

The ADDA group is present in most MCs, as well as nodularins. This chemical group has been used to raise antibodies against these toxins in the development of the commercial enzyme-linked immunosorbent assays (ELISA) used for the detection of microcystins – and nodularins – in water (Fischer et al. 2001).

It is well known that contact with cyanobacteria cells may cause itchy rashes, eye irritation, and other hay fever-like upper respiratory symptoms; however, it is not clear whether these effects are due to the whole microcystin molecule or just a part of it. Moreover, a recent work has hypothesized that MCs could inhibit or reduce the allergic reactions caused by contact with other more allergenic peptides of the cyanobacteria cells (Geh et al. 2016).

Regarding MCs' metabolism, the group in position 7 (opposite ADDA) has been characterized as being involved in the GSH conjugation for toxin excretion (Testai et al. 2016).

Molecular Mechanisms of Toxicity

Evidence concerning the absorption dynamics, the metabolism, and the mechanism of action of microcystins (ADME) derives from experiments performed in animal models (in vivo assays) and conducted on established cell lines (in vitro assays). So far, there are very few epidemiological reports, probably due to the lack of specific biomarkers for microcystin exposure.

Clinical features associated with chronic exposure to microcystins are closely related to the exposure route and metabolism. After being absorbed and incorporated into the bloodstream, these toxins enter any cell, expressing any of the organic anion transporting polypeptides (abbreviated as OATPs) on its surface (Fischer et al. 2005;

Chen et al. 2016), including cells from the kidneys, brain, reproductive organs, and liver, the latter being one of the most widely affected organs, according to the specific literature.

From an experimental perspective, Heinze (1999) explored the effects of chronic intake of contaminated water during 28 days in a murine animal model, using two different doses of MC-LR (50 μg/kg and 150 μg/kg), and demonstrated that in these conditions several hepatic enzymes (ALP and LDH) are altered, as well as the general weight of each animal and the weight of their liver. Also, livers from affected animals had pronounced hemorrhagic necrosis and a considerable accumulation of Kupffer cells. These results have been reinforced by several research groups who analyzed the utility of established cell lines to gain knowledge on the toxicity mechanism of microcystins. For example, Menezes' group (Menezes et al. 2013a) demonstrated the utility of two hepatocyte cell lines – the AML12 (hepatocytes from *Mus musculus*, ATCC-CRL 2254) and the HepG2 cell line (human hepatoma, ATCC-CRL 10741) – and a renal cell line, the Vero-E6 (kidney epithelial cells from the African green monkey, *Cercopithecus aethiops*, ATCC-CRL 1586). In those experiments, the authors found a dose-dependent toxic effect, which consists of a reduction of cell viability, induction of apoptosis, activation of autophagy mechanisms, relocalization of GPR94 – an indicator of stress of the rough endoplasmic reticulum – and the disruption of lysosomes and mitochondria (Menezes et al. 2013b).

All the specific literature agrees that the fundamental mechanism – though not the only one – of microcystin toxicity depends on its ability to inhibit serine and threonine phosphatases PP1 and PP2A (Carmichael 1994). This inhibition leads to hyperphosphorylation of many regulatory proteins that are key to signaling transduction and intracellular metabolism. Considering that these phosphatases are involved in numerous intracellular metabolic routes, the wide range of processes that are affected by microcystins should come as no surprise, nor should the many organs that are compromised during intoxication thereby. Moreover, this pleiotropism is also explained by the ubiquity of OATPs, which is illustrated further in this chapter.

Alterations of Cell Cycle

In terms of the molecular mechanisms of toxicity, McLellan and Manderville (2017) indicated that the inhibition of PP1 and PP2A alters the regulation of the mitogen-activated protein kinase family (MAPK, which includes p38, JNK/SAPK, and ERK). Their activity is related to cell functions like proliferation, differentiation, mitosis, cell survival, and apoptosis.

The authors also suggest that the inhibition of PP2A and alteration of MAPK routes might lead to the disruption of cell cytoskeleton, as others have demonstrated in a human hepatocyte cell line (HL7702) (Sun et al. 2011), and might be the mechanism for the reported oncogenic effect of microcystins.

According to Campos and Vasconcelos (2010), the oncogenic effect of microcystins mentioned by McLellan might be accounted for by the inhibition of PP1 and PP2A, which reduces the activity of DNA repair systems and activates the calcium-

calmodulin-dependent protein kinase II (CaMKII), a protein known to promote cell apoptosis. In their paper, the authors point out that those effects take place indirectly through the alteration of MAPK – modifying its ability to regulate the expression of several proto-oncogenes related to transcription, cell growth, and differentiation – as well as directly through the alteration of proteins related to the cell cycle, like p53, Bcl-2, and Nek2. For example, it is known that the holoenzyme PP1 binds to Nek2, impairing its role as a regulatory protein kinase of the cell cycle, regarding the progress of phase M and during chromosome segregation at the cell division stage, and that PP2A directly inhibits the activity of p53 through the dephosphorylation of its residue threonine-55 (Li et al. 2007). The p53 protein has a central role as a transcriptional transactivator during DNA repair, apoptosis, and tumor-suppressor pathways. PP2A also inhibits Bcl-2, a protein with antiapoptotic activity. In this context, inhibition of PP1 and PP2A would lead to a lack of control of the cell cycle, which might in turn lead to uncontrolled gene expression, proliferation, and cell differentiation.

Toxicity on Organs

Regarding the toxicity on organs, the paper of McLellan points out that – besides the effect of microcystins on PP1 and PP2A – microcystins also react directly with thiol groups, a fact that has been known for years to be related to hepatic toxicity, as in the case of acetaminophen toxicity. On the other hand, several authors indicate that the damage reaches organs other than the liver, like the kidneys, heart, and reproductive organs, matching the known distribution of OATPs (Chen et al. 2016; Milutinović et al. 2006; Roth et al. 2012; Wang et al. 2008).

The distribution of OATPs might help to explain that 50–70% of the MC-LR, when administered intravenously in animal models, is found in the liver and only 1–5% in kidneys and less than 1% in other organs. After the first few hours, only 15% of the toxin is eliminated through feces, and 9% can be found in urine (Robinson et al. 1991).

Several reports in recent decades have been published regarding cases of toxicity or allergenicity by algal blooms, like the work by Cohen and Reif (1953), who described the case of allergic dermatitis to phycocyanin in the case of a 6-year-old girl suffering from skin rash after bathing in a lake.

In Vivo Studies

Concerning in vivo studies, attention should be given to the research by Milutinović, who characterized the renal toxicity of cyanotoxins in a rat model, after repeated sublethal i.p. doses, demonstrating the existence of histological alterations and cytoskeletal disruption (Milutinović et al. 2002, 2003), and the paper by Nobre et al. (1999), who proved the alteration of renal function in a rat model of isolated kidney after the administration of cyanotoxins in doses comparable to an acute intoxication. Other authors explored the effects of MC-LR on a diverse range of organs, tissues, and doses, like Botha – who investigated the effect of a 75% LD50 on different portions of the gastrointestinal tract in a murine model,

showing the increase in the apoptosis rate in the duodenum, jejunum, and ileum in mice (Botha et al. 2004). Concerning this tract, Kubickova et al. (2019) published a review in which they point out different toxic effects of cyanotoxins that were further confirmed by other authors, like the erosion of the small intestine in mice (Ito et al. 2000) and the disruption of cell cohesion in fish (Djediat et al. 2010). In 2003, Shen et al. analyzed the effects of several doses of cyanotoxins on the phagocytic and proliferative functions of the immune system cells in mice, and in 2016, Lone et al. proved the alteration of hematological parameters of mice exposed to microcystins, like leukopenia, thrombocytopenia, and the decrease in the red cell count, hematocrit, mean corpuscular hemoglobin, mean corpuscular volume, and mean corpuscular hemoglobin concentration (MCH, MCV, and MCHC, respectively). Regarding the incidence of neoplasia, Svirčev et al. (2009) and Hernández et al. (2009) analyzed the prevalence of liver and colorectal cancer in human beings from an epidemiological perspective, and their statistical association with chronic exposure to water with algal blooms in Serbia and China, and found a positive and worrying correlation.

In Vitro Studies

In vitro, as stated in the section concerning molecular toxicity, the ability of microcystins to produce cytotoxicity has been extensively documented for diverse cell lines, like adenocarcinoma (human CaCo-2), astrocytoma (IPDDC-A2), and lymphoblastic cell lines (NCNC), in which an increase has been detected in the production of free radicals of oxygen and damage to DNA (Zegura et al. 2008). The ability of MC-LR to reduce general cell viability has also been demonstrated for Vero-E6 (Diaz et al. 2009), among others.

Biomarkers

Definition and Applications

Considering the abundant and growing scientific evidence about the damage caused by chronic exposure to microcystins, and contemplating the diversity of direct and indirect effects that these toxins have on cells and organs, there is a justified need to find strategies to diagnose the exposure to these toxins, based on reliable indicators.

There are several definitions of biomarkers, which have been discussed elsewhere (Strimbu and Travel 2010). In the field of toxicology, these can be classified in several subgroups according to their use, although a single biomarker can be included in many subgroups. These groups are *exposure* biomarkers, when used to confirm or detect contact with any external agent, substance, or factor; *effect* biomarkers, which include any that can be used to evaluate the response of the organism to a medication, treatment, or external agent; and *susceptibility* biomarkers, when used to evaluate the tendency and/or the susceptibility of the organism to a given factor (Gupta 2014).

Biomarkers of Microcystin Exposure

In studying the effects of microcystin exposure and considering the classification mentioned above, one of the most important challenges is to identify biomarkers that make it possible to confirm the exposure to this group of toxins and to diagnose their toxic effects. So far, several biochemical parameters have been proposed, like hepatic enzymes and metabolites (AST, ALT, FAL, GGT, bilirubin) (Li et al. 2011; Zhao et al. 2020), renal parameters (urea, creatinine), and hematological parameters (WBC, PLT, RBC, hematocrit, MCH, MCV, MCHC), as well as the direct detection of the toxin or its metabolites in the bloodstream. In terms of observable signs, it has also been proposed that cutaneous manifestations, allergic symptoms, and acute respiratory manifestations might be markers of intoxication with microcystins.

While biochemical parameters and observable signs are unspecific and not exclusively linked to microcystin exposure (van der Merwe 2014; Chorus and Testai 2021), thus impairing their use as specific exposure or effect biomarkers, direct toxin detection requires specific equipment and laboratory conditions (Chen et al. 2009; Heussner et al. 2014), and its sensitivity is conditioned by the speed at which the toxin is cleared from the bloodstream and rapidly distributed to the liver, kidneys, and other organs, or eliminated by feces and urine. As an example of the complexity of the situation, Sedan et al. (2013) showed the modification of biochemical parameters in a murine model under chronic exposure to MC-LR; the authors observed alterations in the level of ALT, albumin, bilirubin, methemoglobin, glutathione, and α-tocopherol, together with modifications in the production of free radicals of oxygen, the SOD activity, and the lipidic profile. Nevertheless, the only binding parameter these authors found with MC-LR exposure was the detection of MC-LR in the bloodstream.

Taken together, all this evidence reinforces the need for proper biomarkers, which would allow for adequate diagnosis and propitiate proper epidemiological surveillance and population studies.

Antibodies as Biomarkers

Immunoglobulins (Igs) are proteins produced by activated B lymphocytes (aBL). They are heterotetramers composed of four polypeptides: two heavy chains (H) and two light chains (L). The antigen-binding site – called paratope – is composed of peptide loops belonging to both H and L chains, and as these chains are identical, each Ig has two paratopes (see Fig. 2). Each aBL produces Igs with a single specificity, although the constant regions that determine the isotype can vary in a process named *isotype switch* (see the next section). Once the switch has occurred, it cannot be reversed (Chiu et al. 2019; Schroeder and Cavacini 2010).

Knowing the antigen under study, the presence of specific Igs can be studied in different biological fluids, and detecting it provides information regarding whether the organism has been in contact with that antigen before. Besides the participation of specific Igs – called antibodies (Abs) – in the immune response to that antigen, the

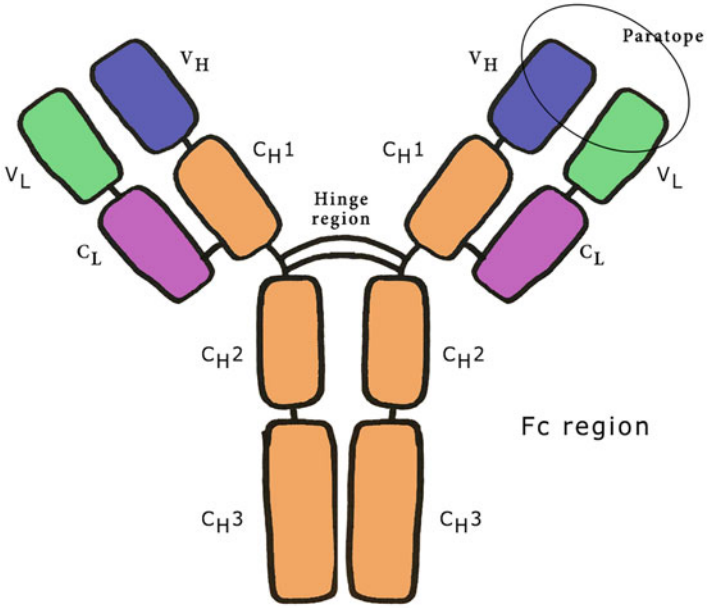

Fig. 2 General structure of immunoglobulins. VH and VL are variable domains of heavy and light chains; CH1, CH2, and CH3 are the constant domains of the heavy chain; and Fc is the name of a region composed of the CH3 domain and part of the CH2 domain

detection of specific Abs can be considered an indicator of the immune response and thus a marker of a prior contact with the antigen.

Specific Abs have been extensively used as biomarkers, as in autoimmune diseases, oncological processes, and infectious diseases. Autoantibodies, which are Igs that recognize self-structures as a consequence of the loss of self-tolerance, are present both in classic autoimmune diseases like lupus or rheumatoid arthritis, conditions in which Abs are also responsible for the clinical symptoms, and cancer, cardiovascular, and neurodegenerative diseases (Aziz et al. 2018).

Fässler et al. (2019) demonstrated that Abs associated to melanoma – IgG specific for tyrosinase-related protein types 1 and 2, glycoprotein 100 (gp100), MelanA/MART1, and the testicular antigen NY-ESO-1 – are good markers of the response of the organism to the treatment with cell-cycle checkpoint inhibitors. With a similar logic, Tikhonov et al. (2020) pointed out that a specific profile of Abs is raised due to abnormal glycosylation, which in turn makes it possible to classify the tumor.

Moreover, Yanamandra et al. (2011) proposed specific Abs to α-synuclein as diagnostic biomarkers for diseases that are characterized by neurodegeneration, like Parkinson's disease, while Srivastava et al. (2012) pointed out the presence of antibodies to potassium channel KIR4.1 in almost 47% of the patients with multiple sclerosis.

The utility of Abs as diagnostic biomarkers in the field of infectious diseases is historical and goes beyond this revision. It has been extensively documented for

viral, bacterial, and parasitic pathologies like HIV, hepatitis, syphilis, and Chagas disease, in which it can be used to distinguish acute from chronic conditions, taking advantage of the isotype switch of specific antibodies raised against the infectious agents.

Concerning Abs as biomarkers for intoxication with natural toxins, there are few reports describing their use and performance in diagnosis (Khojasteh et al. 2003; Lefebvre et al. 2012). The reasons for this lack of information might be that most of the toxins lead to acute conditions in which the adaptive immune system has not enough time to produce specific antibodies.

Isotype Switch of Antibodies

The immune response can be classified as primary, when the antigen first enters the organism, and secondary, when the immune system interacts with it in any further encounter. During a primary Ab response, the dominating antibody isotype is IgM; this Ig has a pentameric structure composed of relatively low-affinity subunits. Because of its large size, IgM can be found mainly in the bloodstream and can rapidly bind antigens and activate the complement system. Microbial carbohydrates mostly lead to the production of specific IgM, although this isotype also recognizes proteins.

During a secondary response, Abs switch from IgM to any of the other Ig isotypes, depending on the biochemical nature of the antigen and the entrance pathway to the organism. For example, IgG is the most abundant isotype in the bloodstream, and in order for the antigens to be found by these Abs, they must first transpose barriers like the respiratory mucosa or the digestive tract. These Abs are high-affinity monomers and increase their affinity due to a hypermutation mechanism that occurs at the DNA level of plasmocytes – the activated B lymphocytes. Moreover, the isotype switch also leads to the establishment of a subset of B cells called memory cells, which will be responsible for Ab production whenever the antigen enters the organism again. In that case, IgG production will be faster and more intense; IgG has a considerable ability to activate the complement system and to bind Fc receptors of phagocytes, thus helping the immune system to remove the antigen from circulation.

The production of IgA is associated with mucosal tissues and salivary fluid. This Ig circulates as a dimer and is particularly produced to be secreted in the extracellular space mucosal tissues. Tissue locations where IgA can be found are mostly devoid of phagocytes and complement proteins; thus, IgA functions are not directly related to antigen depuration but to antigen neutralization.

The IgE isotype is mainly found associated with mast cells via their Fc surface receptor and, to a much lesser extent, free in the bloodstream. Antigen binding to associated IgE leads to mast cell degranulation, releasing biochemical mediators of inflammation and chemotaxis. Thus, this isotype is associated with allergy because the immune response to common allergens leads to “silent” progressive sensitization of mast cells with specific IgE molecules, which in turn might produce a massive

degranulation when the antigen enters the organism again. Degranulation of mast cells leads to common clinical manifestations of allergy, like respiratory compromise, generalized inflammation, edema, etc.

In this context, determining the isotype of specific antibodies gives information concerning the entrance pathway of the antigen and also about the number of times the antigen has entered the organism (single recent contact, repeated past contact). Furthermore, the correlation between the antibody isotype and clinical features developed by individuals might make it possible to understand the ability of immunoglobulins to protect the organism from any particular antigen, either virus, bacteria, or toxic compounds.

Antibodies Against Microcystins

The use of specific antibodies as biomarkers for waterborne infectious diseases has been documented elsewhere, like the paper by Exum et al. (2016). In that work, the authors point out the utility of detecting pathogen-specific antibodies during the identification of the causal agent of gastrointestinal disease caused by norovirus, **shiga toxin-producing** *E. coli*, *Cryptosporidium* spp., rotavirus, *Shigella* spp., *Giardia* spp., *Vibrio cholerae*, *Campylobacter* spp., hepatitis A, and hepatitis E.

The presence of specific antibodies against microcystins in individuals under chronic exposure to contaminated waters has not yet been conveniently studied. The lack of evidence concerning this issue is so marked that there is still no information on whether microcystins in the bloodstream – which are small molecules and probably behave as haptens – bind any carrier protein to become immunogenic. This question is still unanswered, although the molecular structure and some laboratory assays (Meissner et al. 2013) suggest that lateral groups of amino acids might be reactive and produce a covalent bond to tissues or plasma proteins, like albumin. The production of hyperimmune sera specific for MC-LR in laboratory animals suggests that conjugation with carrier proteins is needed for immunogenicity (Baier et al. 2000; Metcalf et al. 2000; Mhadhbi et al. 2006; Young et al. 2006) and that the antibodies obtained in this manner might provide protection against toxins (Nagata et al. 1995). Although these are experimental conditions, data suggests that conjugation to carrier proteins might occur spontaneously at the physiological pH, giving rise to a specific immune response.

In terms of the utility of specific antibodies as exposure biomarkers for the exposure to microcystins, to our knowledge, there is only a single published paper so far, which belongs to our research group. In that paper, a group of settlers near a lake with recurrent algal blooms were first studied using a questionnaire in order to evaluate the degree of exposure and the exposure routes, and then the alteration of biochemical parameters and the presence of specific antibodies were studied in order to provide experimental evidence of that exposure. In that paper, we demonstrated that although the alteration of the hepatic enzymes was detected in only 25% of the exposed individuals, the presence of specific antibodies against MC-LR – either IgG or IgE – could be positively used to distinguish the exposed population from a

control group (Ruibal-Conti et al. 2019). Regarding antibody values, the exposed/non-exposed ratio was 7:1 for IgE and 3:1 for IgG, thus indicating that these molecules are good biomarker candidates for the identification of MC-LR exposure.

Applications to Prognosis

In this chapter, we have reviewed the molecular structure of microcystins and the biological mechanism of their toxicity. Furthermore, we have reviewed their distribution, target organs, and their effects in terms of the alteration of biochemical parameters, analyzing literature presenting in vivo and in vitro-based experimental evidence (see Fig. 3).

Considering the need for the identification of suitable biomarkers for assessing chronic exposure to microcystins, we here discussed the use of specific antibodies to microcystins and presented the available evidence. In particular, we reviewed the only experimental paper published on this matter, published by our research group (Ruibal-Conti et al. 2019), in which the detection of specific IgG and IgE against MC-LR proved to have a stronger correlation with the exposure status of the population under study than classic biochemical parameters like hepatic enzymes.

The use of specific Abs against microcystins as exposure biomarkers may have several advantages, among which are the fact that antibody detection is relatively simple and that it could be used to distinguish acute from chronic exposure (e.g.,

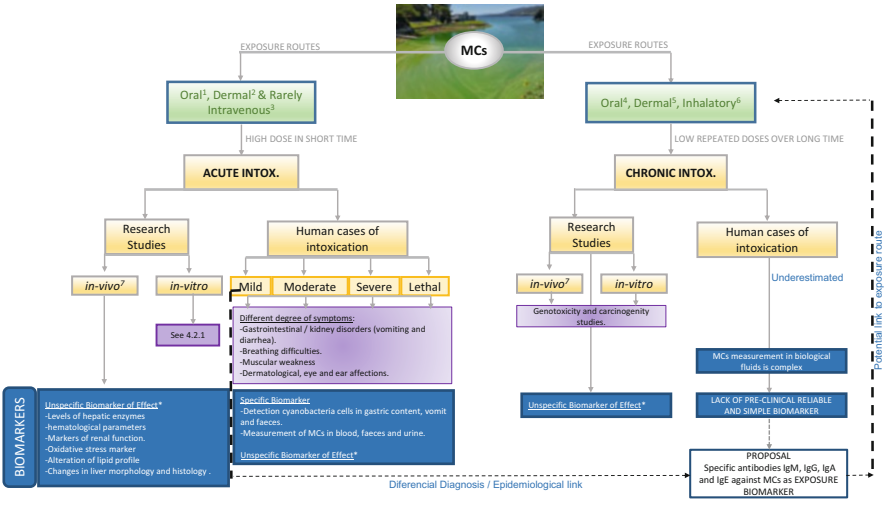

Fig. 3 General scheme for exposure routes to MCs, symptoms of acute or chronic intoxication, and potential biomarkers. 1: Generally accidental ingestion. 2: Generally by recreational or work-related skin contact. 3: Caruaru episode. 4: Generally through contaminated food, dietary supplements, or drinking water. 5: Generally by recreational, occupational, or domestic skin contact. 6: Generally inhalation of aerosols during prolonged or recurrent nautical activities or extended residence in the area. 7: Generally experiments using high doses and intraperitoneal addition of MCs

IgM in acute cases, IgG in chronic cases) if a correlation is confirmed between the isotype and exposure time. Furthermore, Abs might constitute adequate tools for the implementation of population studies and epidemiological surveillance programs.

The main obstacles related to the use of specific antibodies as biomarkers of exposure to microcystins are of a theoretical and technical nature. On one hand, there is still a considerable lack of information concerning how the immune system reacts to toxins and to what extent the exposure route shapes that response. Although the standard IgM/IgG scheme to discriminate acute from chronic conditions works for many infectious diseases, an algorithm including IgA, an isotype usually predominant in mucosal tissues, like the intestine or oral cavity, or including IgE, an isotype that is associated to allergic responses, should not be discarded. Concerning technical obstacles, it should be stated that culturing toxin-producing cyanobacteria in laboratory conditions is a delicate procedure with many difficulties and that toxin purification is a key process when designing assays for antibody detection, because any microbial contaminant is likely to produce interference in the detection system, leading to cross-reactions that impair further analyses.

Taken together, all this evidence suggests that Abs are suitable candidates for constituting exposure biomarkers of exposure to microcystins – and potentially other cyanotoxins – although further research is still needed to assess, for example, whether their titration could provide more information, and the duration of these molecules in the bloodstream or biological fluids. Moreover, there is still much research to be done regarding their protective capacity to prevent the development of clinical symptoms of intoxication.

Mini-dictionary of Terms

- **ADDA**: Unusual non-protein β-amino acid that is part of the chemical structure of microcystins and is involved in the toxicity mechanism. It is found in all microcystin congeners and also in nodularins.
- **Antigen**: A structure "foreign" to the organism that can be detected by the immune system.
- **Cyanobacteria**: Ancient unicellular microorganisms related to gram-negative bacteria but with green and blue pigments that make them capable of photosynthesis. They represent the earliest known form of life on the earth.
- **Cyanobacterial bloom**: Massive development of cyanobacteria that turns the color of water intense green.
- **Paratope**: Part of an antibody that recognizes and binds to an antigen.
- **Isotype**: Subclass of immunoglobulins.
- **Antibody**: Proteic immunoglobulin, part of the adaptive immune response.
- **Glycosylation**: Specific cell-associated pattern of surface carbohydrates.
- **Hypermutation mechanism**: A somatic mechanism in which some genes vary during cell proliferation.
- **Fc**: Constant fragment "c" of immunoglobulins.
- **Chemotaxis**: A process by which immune cells move toward their site of action.

- **Massive degranulation**: When mast cells release their secretory vesicle content.
- **Leukopenia**: A condition in which there is a lower-than-normal number of leukocytes (white blood cells) in the blood.
- **Hapten**: A "foreign" particle that cannot start an immune response de novo.
- **Thrombocytopenia**: A low platelet count.

Key Facts of Antibodies as Biomarkers: Effect of Microcystin Exposure

Key Facts of Cyanobacterial Blooms

- Cyanobacterial blooms are not always toxic. However, for risk management strategies, any cyanobacteria bloom is considered potentially toxic until toxicity is tested.
- Microcystins are secondary metabolites not excreted by cyanobacterial cells; they are released into the surrounding waters when cyanobacteria cells disintegrate due to natural or artificial adverse conditions.

Key Facts of Microcystins

- Microcystins are stable, thermoresistant, but photolabile molecules with a typical environmental half-life of 10 weeks.
- Microcystin can bioaccumulate in fish muscle and viscera.
- Microcystin-LR is classified IARC 2B, which means "possibly carcinogenic to humans," by the International Agency for Research on Cancer.

Key Facts of Human Intoxications with Microcystins

- The most relevant case of microcystin intoxication occurred in 1996 in a hemodialysis unit in Caruaru, Brazil, where 60 patients died.
- There are currently no specific treatments, antidotes, or vaccines.
- Treatment is based on decontamination if exposure is recent, supportive measures, maintenance of a patient airway, and symptomatic treatment.

Summary Points

- Toxic cyanobacteria growth in water bodies is a worldwide problem, and the concern it generates is genuine and growing.
- Microcystins are small cyclic heptapeptides produced by cyanobacteria and characterized by an ADDA group that is partly responsible for the toxicity of the molecule and might be involved in its immunogenicity.
- At the cellular level, MCs inhibit serine/threonine protein phosphatases 1 and 2A affecting many metabolic pathways, in particular multiple control mechanisms of the cell cycle.

- Microcystins are mainly hepatotoxic but may affect organs as diverse as the kidneys, nervous system, respiratory system, and reproductive organs.
- Health effects of MC-LR have been proven in cell lines and animal models, and worrying statistical associations have also been reported in epidemiological studies.
- The clinical effects of microcystin exposure depend on the route and the level of exposure, ranging from mild, severe, to lethal.
- Acute exposure to high concentrations of these toxins causes liver damage, while subchronic or chronic exposure may promote liver or colon tumor formation.
- Acute intoxication with MCs is difficult to diagnose because its symptoms and effect biomarkers are nonspecific and easily confused with those of other diseases.
- Chronic or subchronic intoxication with MCs is difficult to identify as there is no specific preclinical biomarker.
- Proposed effect biomarkers are unspecific and include hepatic enzymes, hematological parameters, oxidative stress markers, and markers of renal function.
- Isolation of the toxin in blood and tissue is complex and requires the use of sophisticated equipment.
- The use of specific antibodies as biomarkers for waterborne infectious diseases is well known but has not yet been conveniently studied in the field of toxic cyanobacterial blooms.
- Theoretical evidence indicates that antibodies against MC would be a useful, simple, and specific method/biomarker to measure or follow up exposure to microcystins, although the only existing experimental evidence is from work done by our group.
- Antibodies include different isotypes that could be indicative of the type of exposure route.
- In the future, it will be necessary to investigate the link between the different immunoglobulin isotypes and the titration and persistence of antibodies over time in order to reliably establish the potential of specific antibodies as biomarkers of exposure to cyanotoxins.

Cross-References

► Biomarkers for Assessing Mycotoxin Exposure and Health Effects
► Biomarkers of Liver Injury due to Toxic Agents: Progress, Current Applications, and Emerging Directions

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