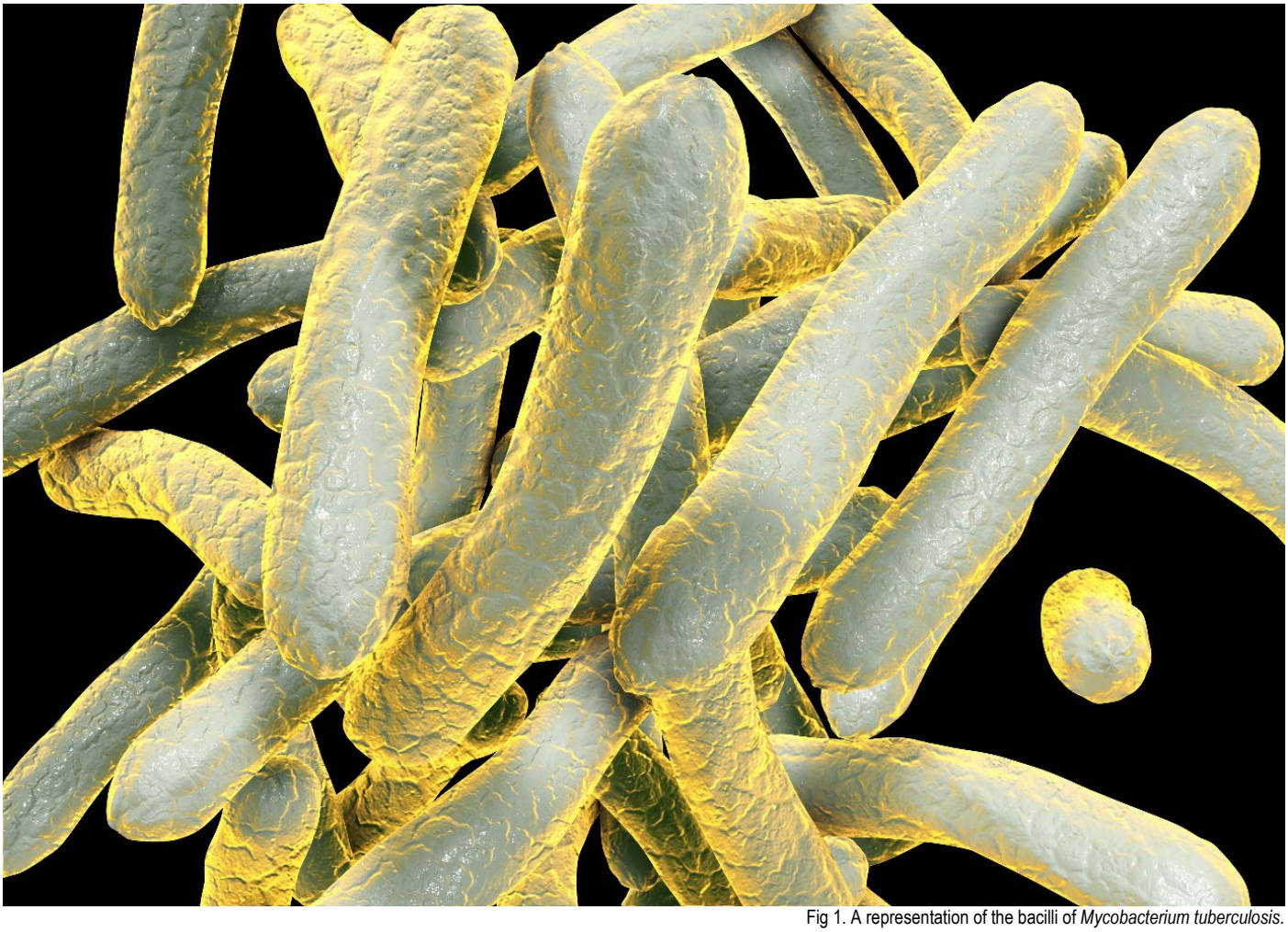

Fig 1. A representation of the bacilli of *Mycobacterium tuberculosis*.

Finfish Diseases: Mycobacteriaceae: Nontuberculous Mycobacteriosis (NTM)

Chris Aslett

Before launching headlong into an exploration of Mycobacteriaceae, we explore the rudiments of bacterial behaviour, anatomy, and some of the family's interactions with vertebrate defence.

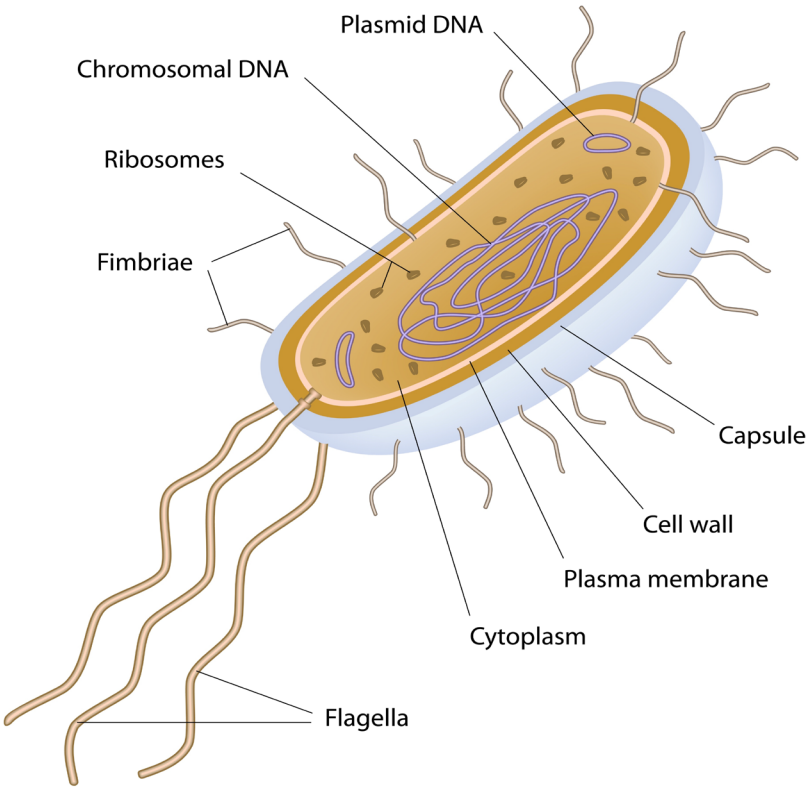

Fig 2. A diagram of a bacterium which lacks a nuclear membrane and complex organelles, yet it comprises ribosomes, flagella, a concise chromosome of genomic DNA, and circular DNA known as a plasmid.

Prokaryotic [*pro*: "before"; *karyo*: "nucleus"] bacteria are typically unicellular free-living organisms which lack the complexity and numerosity of eukaryotic organelles, whilst their concise DNA genomes are merely supercoiled into a simple chromosome which is not enveloped by a nuclear membrane (Figs 2. & 3.; Griswold 2008).

Copious varieties of marine bacteria are small, flagellated, and motile (Macnab & Aizawa 1984; Fenchel 2002), whose swimming appears at first to be disorganised tumbling (Gluch et al. 1995). However, their travel (taxis) can occur along a gradient of magnetism (magnetotaxis), sunlight (phototaxis), temperature (thermotaxis), or chemicals (chemotaxis; Kihara & Macnab 1981; Blakemore 1982; Gest 1995; Gluch et al. 1995). Cell-surface receptors detect a stimulus which is transduced into a proportionate signal that modulates the activity of their flagella motor(s; Figs 2. & 4.). Hence, they positively move towards or negatively away from the stimulus (Lux & Shi 2016). Therefore, pathogenic bacteria may seek the gills of a host by swimming towards ammonia, whereas others may use the daybreak sun to ascend or descend.

Keywords: Mycobacteriaceae; *Mycobacterium marinum*; Nontuberculous Mycobacteriosis; NTM; tropical marine aquarium fish.

The Microbial Consortia of a Fish's Microbiomes

The unique microbiomes of the gills and gut of each reef teleost (finfish) provide a variety of distinct ecological niches for exceedingly diverse but interrelated microbial populations. More alike community profiles are found in members of the same species compared with those of unrelated teleosts or those with comparable diets (Pratte et al. 2018). A remarkable symbiotic relationship was identified within the gills of freshwater fish, because clusters of brachial intracellular bacteria can aerobically transform ammonium (NH_4^+) to dinitrogen (N_2; van Kessel et al. 2016).

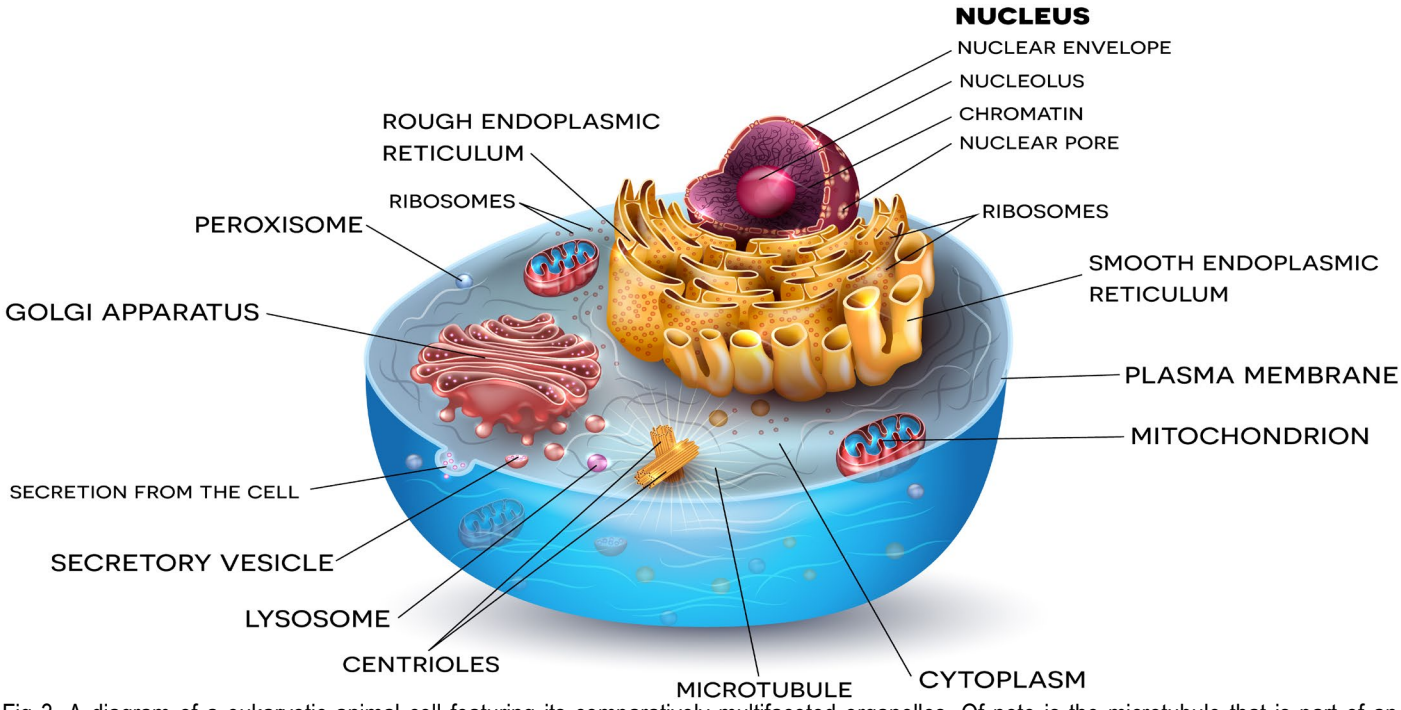

Fig 3. A diagram of a eukaryotic animal cell featuring its comparatively multifaceted organelles. Of note is the microtubule that is part of an intracellular framework called the cytoskeleton composed of filaments of actin and tubulin (Wallace et al. 1996).

Tumour Necrosis Factor Alpha

Multiple antibodies and antigens form immune complexes through the binding and clumping of epitopes and paratopes (Almeida & Waterson 1969) where such agglutinations are engulfed (phagocytosed) by innate-response macrophages which ultimately purges them from the body (Figs 8., 9., 19., 21., & 22.). Likewise, macrophages internalise cellular debris or dying cells and manufacture the signalling cytokine, tumour necrosis factor-alpha (TNF-α). Transmembrane expressed TNF-α becomes extracellular when cleaved by a metalloprotease, which induces necrosis of atypical cells with pronounced genetic aberrations (Chau et al. 2004; Hong et al. 2013). Rainbow trout's (*Oncorhynchus mykiss*') TNF-α is liberated by numerous immune cells including macrophages and T-lymphocytes, whereas the TNF-β of higher vertebrates has yet to be discovered in fish (Laing et al. 2001).

TNF is present in mammals including humans which prevents the development of malignant tumours, yet they can also promote neoplasms (Wang & Lin 2008), where immunostimulation is the "go-to" treatment for cancers (Liu 2007; Yan 2018; Farolfi et al. 2019).

Granulomas

Granulomas are tissue-bound benign cysts (tumours) formed by the disease-causing agent and host defence, where infections are "walled off" with immune cells and collagenous fibroblasts. They represent an acute inflammatory response distinct from nominal infiltrations of "white cells" (Figs 10. to 14.).

Mycobacteriaceae: Taxonomy

Bacteria of the family Mycobacteriaceae previously classified in the genus *Mycobacterium* include *Mycobacteroides abscessus* and *M. chelonae*; *Mycolicibacter arupensis* and *M. nonchromogenicus*, and *Mycolicibacterium aurum*, *M. fortuitum*, *M. parafortuitum*, *M. peregrinum*, and *M. poriferae* (NCBI Taxonomy Browser 2022). Upheld species of *Mycobacterium* comprise *M. avium*, *M. gordonae*, *M. interjectum*, *M. marinum*, *M. szulgai*, and *M. triplex* (Novotny et al. 2004; Zanoni et al. 2008; Zhang et al. 2015). All belong to the kingdom Monera, the clade terrabacteria, the phylum Actinobacteria, the class Actinomycetia, and the order Corynebacteriales (WoRMS 2020; NCBI Taxonomy Browser 2022).

Two members of the genus *Mycobacterium* are conspicuous pathogens of humans: *Mycobacterium tuberculosis* and *M. leprae*, which are the aetiological agents of tuberculosis (TB) and leprosy (Smith 2003; Singh & Cole 2011). Ubiquitous zoonotic *Mycobacterium marinum* (synonym: *Mycobacterium piscium*) participate in granuloma formation in wild and farmed fish (Gauthier & Rhodes 2009; Francis-Floyd 2011). *M. marinum* or *M. leprae* cannot grow above 30 or 35°C; and are thus restricted to surface ulcerations or the peripheral nervous system in most mammals (Ranger et al. 2006; Fukutomi et al. 2009). Although now considered unnecessarily inhumane, the response of nine-banded armadillos (*Dasypus novemcintus*) to experimental challenges with *M. leprae* have proven enlightening, insofar as they have the lowest body temperature of any mammal. They cannot, however, acclimate to greater latitudes.

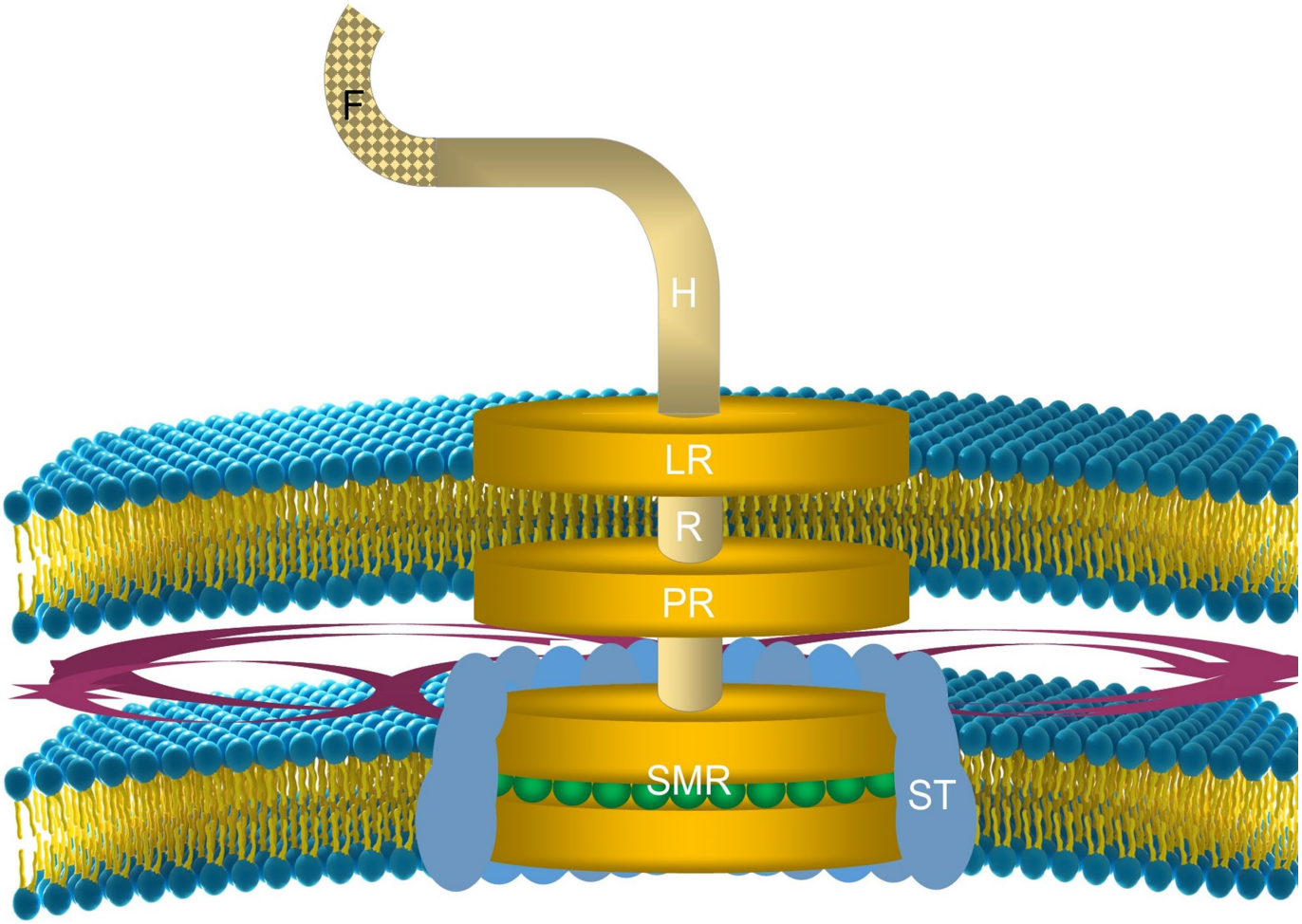

Fig 4. An illustration of the transmembrane flagellum motor of a motile gram negative bacterium: [F] flagellum base; [H] hook; [LR] L-ring; [PR] P-ring; [R] rod; [SMR] S-M-ring assembly, and [ST] stator.

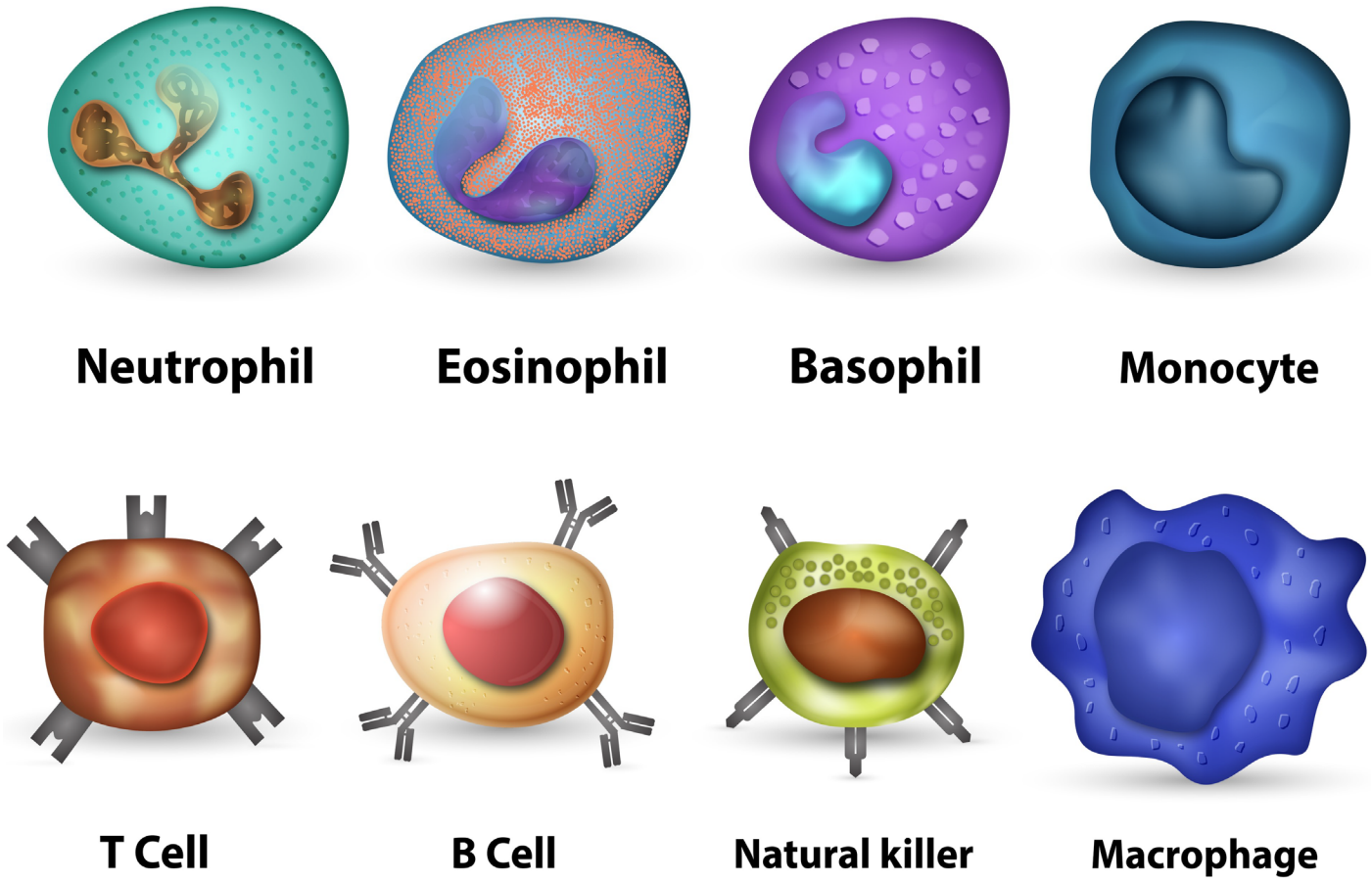

Fig 5. The “white” cells of the immune system: leukocytes, granulocytes, and lymphocytes. Top - the granulocytes of the inflammatory response contain multilobed nuclei and cytoplasmic granules of peroxidase. Bottom - excluding the macrophage, lymphocytes are components of the adaptive response which carry ultraprecise surface receptors.

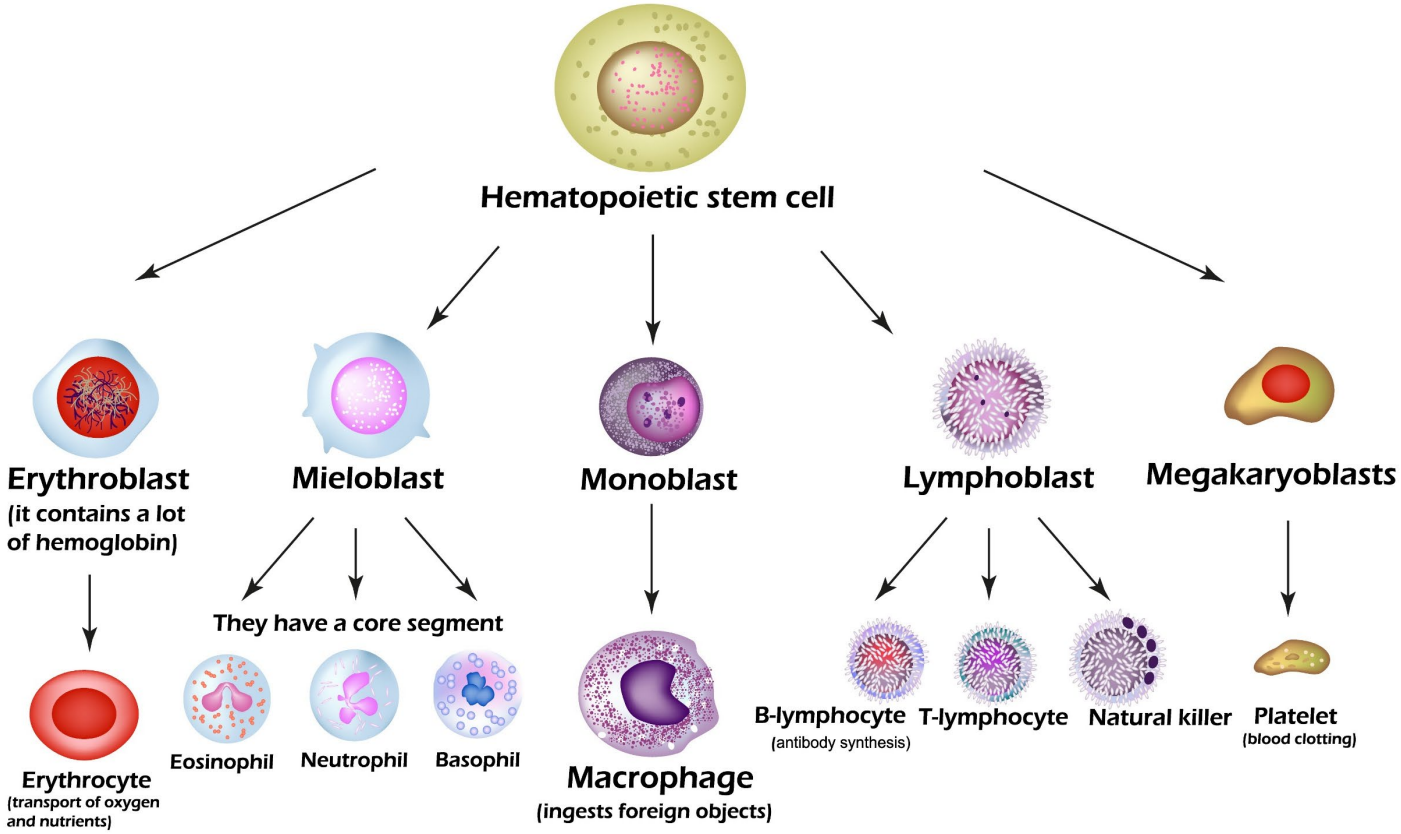

Fig 6. The differentiation potential of a haematopoietic stem cell.

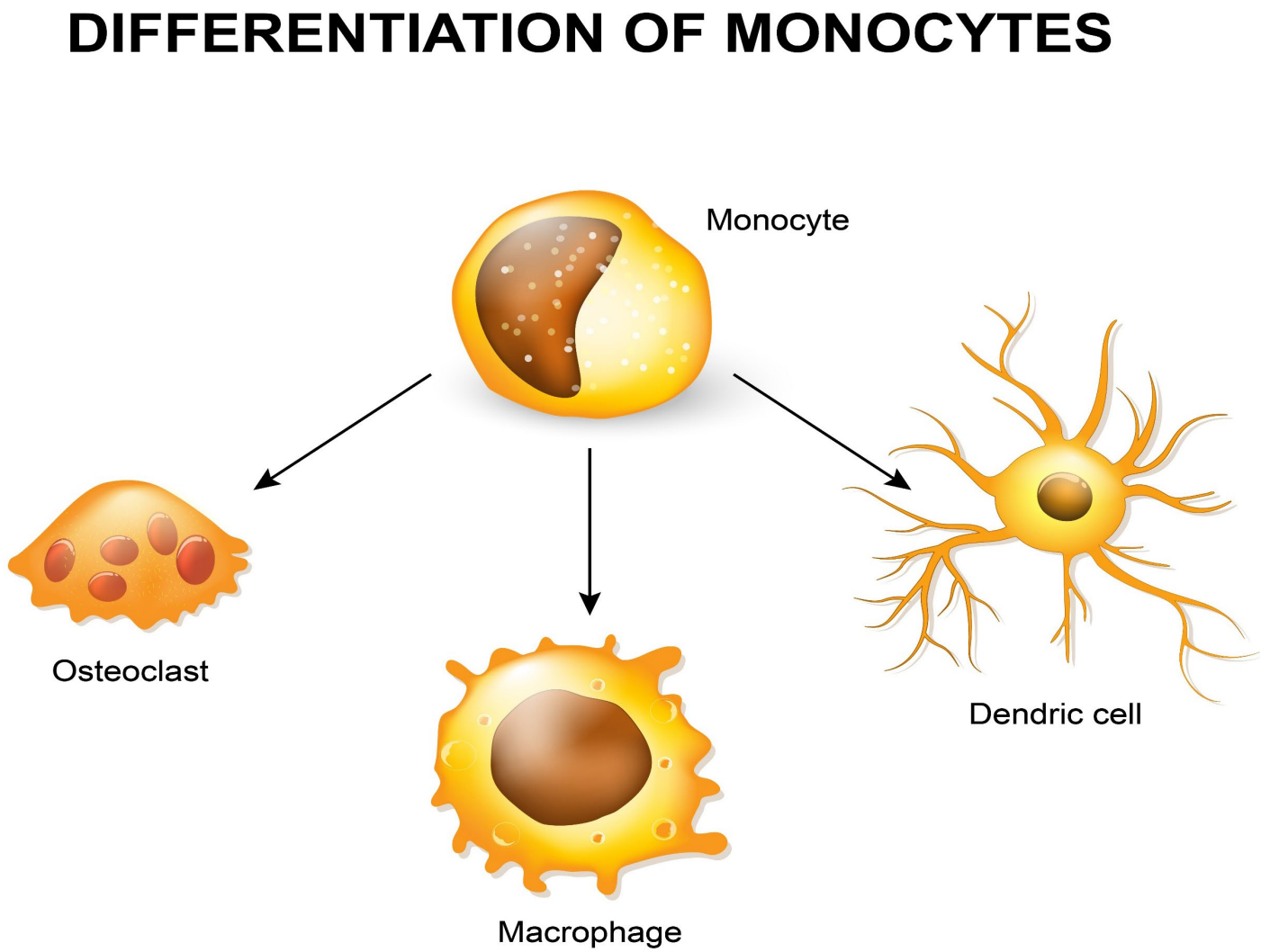

Fig 7. Monocytes (monoblasts; Fig 6.) differentiate to form macrophages, dendritic cells, and osteoclasts which orchestrate bone degradation throughout pathogenesis and its corrective reconstruction (Chatani et al. 2016), whereas dendrocytes present antigens to both arms of immunity to coordinate antiparasitic responses (Bassity & Clark 2012).

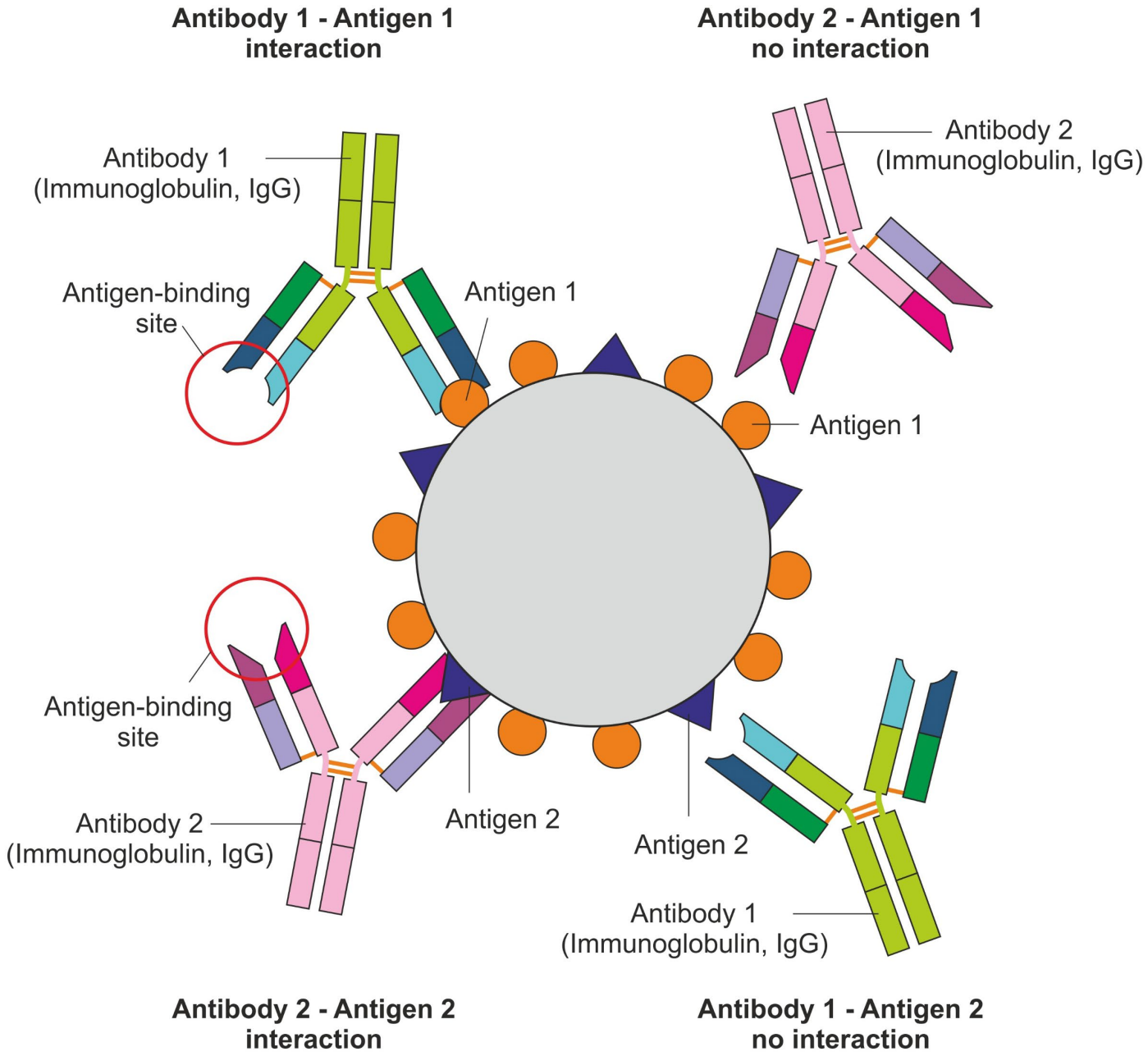

Fig 8. Two kinds of antibody binding to surface antigens in a polyclonal serum.

Like human TB, diseases orchestrated by *Mycobacterium marinum* are punctuated with granulomatous whitish grey nodules (tubercles; Ang et al. 2000; Pro 2003; Francis-Floyd 2011), where surprisingly, fish immunity and microbes contribute to their development (Stoop et al. 2011). The immune system encircles a necrotic focus of infection with macrophages which engulf the *Mycobacterium* and become engorged and enlarged, whereupon leucocyte demise triggers the deposition of a caseous (cheese-like) core replete with live but quiescent bacteria surrounded by mono- and lympho-cytes with fibroblastic cells (Fig 13. left; Fig 16.; Smith 2003; Tobin & Ramakrishnan 2008; Francis-Floyd 2011).

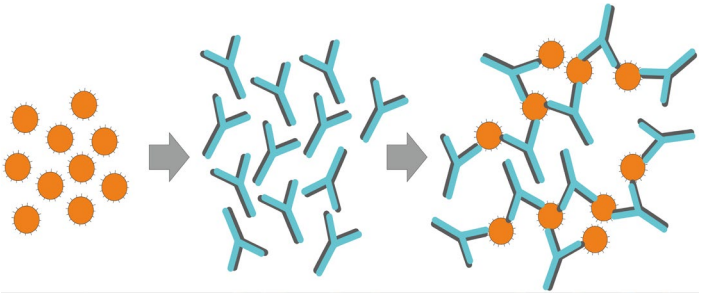

Fig 9. Antibody/antigen agglutination and the formation of an immune complex.

Nontuberculous mycobacteriosis (NTM) was first reported in saltwater fish in 1910 (Von Betegh 1910), whereafter the same kind of microorganism was isolated from marine teleosts that died at the Philadelphia public aquarium (Aronson 1926). *Mycobacteroides chelonae*, *Mycolicibacterium fortuitum*, and *Mycobacterium marinum* remain the most prominent of fish Mycobacteriaceae (Gauthier & Rhodes 2009, cited in Hashish et al. 2018). Acute or chronic disease is determined by exposure, because one million colony forming units per litre (CFU l^{-1}) overwhelm fish and cause mortality, whereas less than one hundred thousand instigate chronic disease (Prouty et al. 2003).

M. marinum are likely ubiquitous intracellular parasites infecting fish in all environments, yet they are currently unrecognised in fish inhabiting colder seas (Reichenbach-Klinke 1972, cited in Roberts 1989g; Pro 2003; Francis-Floyd 2011; Colorni & Diamant 2014), which may, like those infected with *Photobacterium damsela* subspecies *damsela*, become asymptomatic carriers at lower temperatures (Magariños et al. 2001; Toranzo et al. 2005; Colorni & Diamant 2014).

Contagion conceivably occurs via ingestion of infected flesh, vertically from mother to offspring, or through exposure to waterborne suspensions of bacteria at the gill or gut (Diamant et al. 2000; Ortega et al. 2014; Hashish et al. 2018). An incubation phase that lasts around two weeks precedes the onset of clinical signs (Hashish et al. 2018), whilst conspecific susceptibility remains exceptionally varied insofar as disease frequently develops in 10 to 100 percent of fish (Francis-Floyd & Yanong 2002).

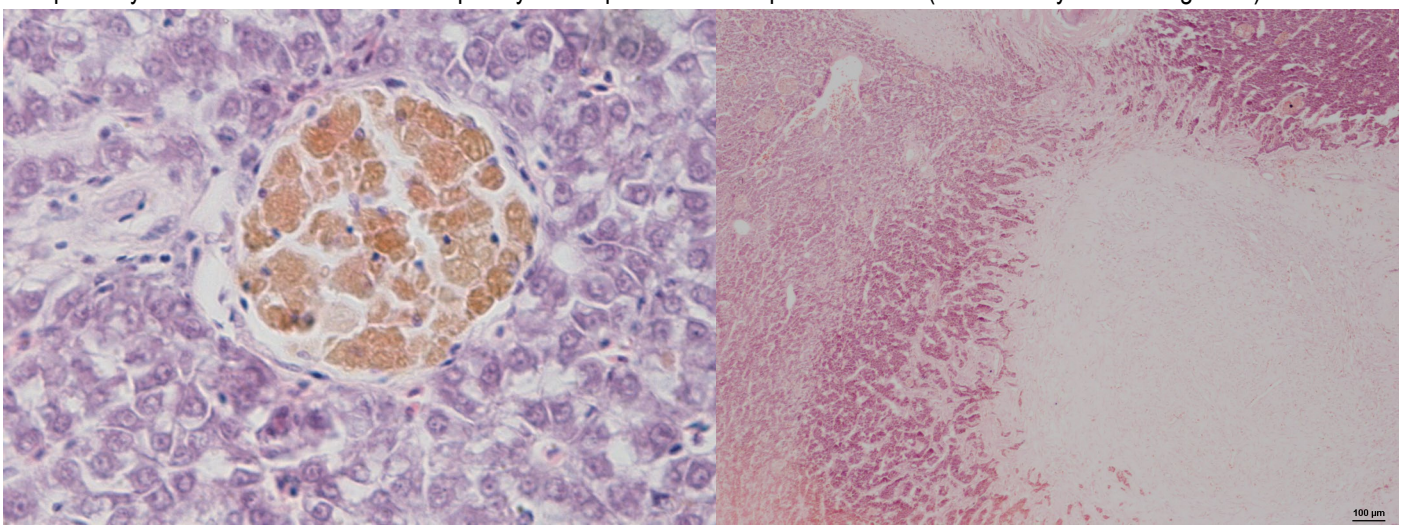

Fig 10. Left - macrophages surrounded by monocytes in the liver of common sole (*Solea solea*) possibly caused by stress. Right - a white soft fibrous mass in the liver of common dab (*Limanda limanda*) infiltrated with macrophages. Neither are "true" granulomas because they lack clear aetiology (idiopathy). Contains public sector information licensed under the Open Government Licence (OGL) v3.0. Consult bibliography.

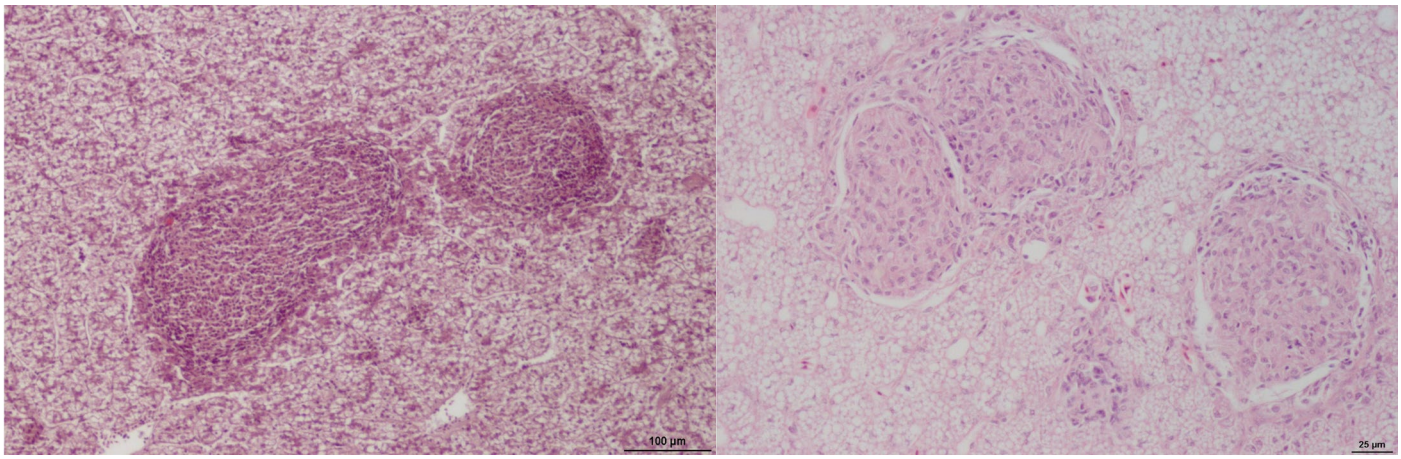

Fig 11. Left - non-granulomatous multiple foci of basophilic hepatocytes in common dab (*Limanda limanda*) typically associated with environmental contaminants, which features inflammation and infiltrations of the pigmented melanomacrophages of poikilotherms (Stosik et al. 2019). Right - granulomas formed in response to gram negative bacteria of the genus *Francisella* which appeared as visceral nodules in Atlantic cod (*Gadus morhua*). Contains public sector information licensed under the Open Government Licence (OGL) v3.0. Consult bibliography.

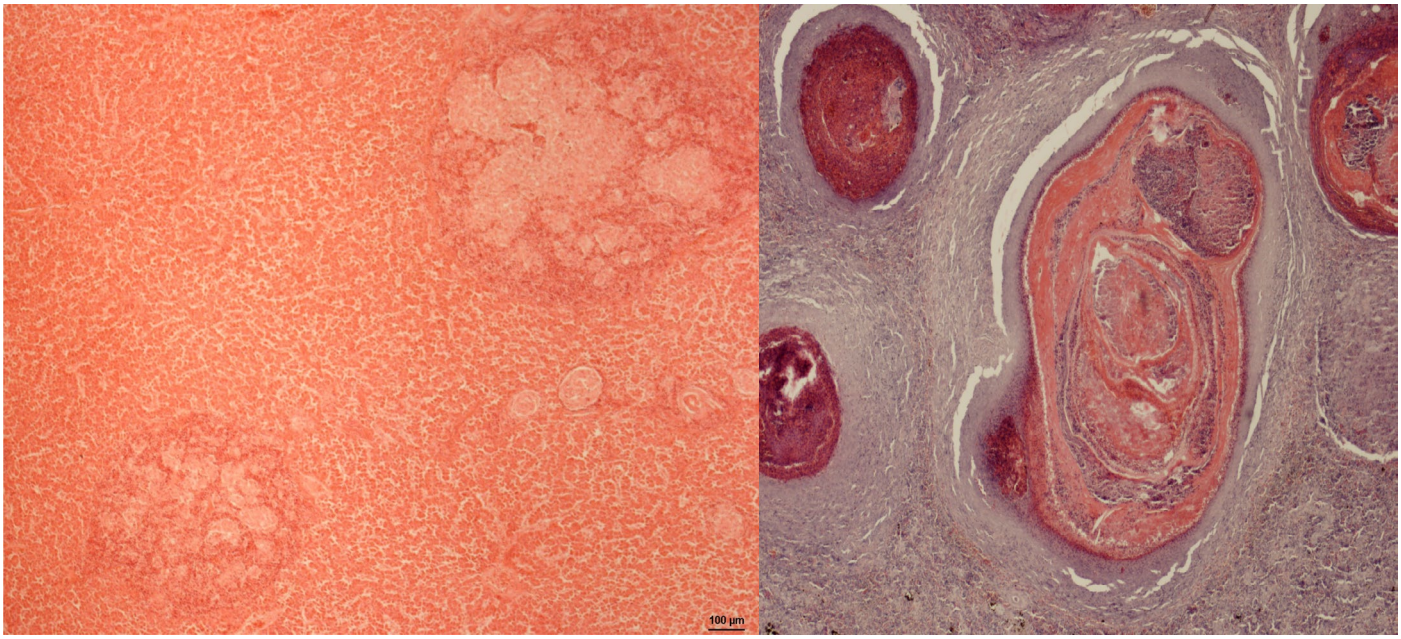

Fig 12. Inflamed multifocal necrotic granuloma in the liver of *Merlangius* species of whiting, likely caused by bacteria of the genus *Francisella*. Contains public sector information licensed under the Open Government Licence (OGL) v3.0. Consult bibliography.

Diagnosis and Genetic Diversity

Cultures and homogenates must be handled with stringent biosafety precautions (Francis-Floyd 2011). Species of *Mycobacterium* are pleomorphic, insofar as they are typically non-spore-forming, aerobic, gram positive nonmotile bacilli, but they may also be coccoid or ovoid that stain inconsistently (Lack & Tanner 2003; Hashish et al. 2018), since gram stains frequently manifest as beads or bars (Roberts 1989d). Each rod is nominally 1 to 4 by 0.2 to 0.6 µm, but 10-µm bacilli are not uncommon (Figs 1. & 15.; Roberts 1989d; Francis-Floyd 2011). Their stains are acid fast because they resist the decolourising properties of acids or alcohols, which is lost in starved cultures (Nyka 1974; Bayot et al. 2020), whereas acid/alcohol fastness is confirmed using Ziehl-Neelsen's methodology (Fig 16.; Roberts 1989d) where a sterile loop from a single colony growing on agar is smeared onto a pre-ethanol dowsed and flamed slide. The film is then left to airdry around the base of a lit Bunsen after which it is fixed by briefly passing it twice through the blue part of the flame with tongs. Slides are suspended smear side upwards on glass rods over a stainless steel or porcelain sink and flooded with carbol fuchsin then heated from beneath until they begin to liberate steam without boiling. After three to four minutes, the slides are reheated and then left a minute or two before thoroughly rinsing under running water. The film is then repeatedly decolourised with dilute sulphuric acid and ethanol where the lack of pigment is appraised microscopically. Slides are allowed to airdry after a minute's counterstain with malachite green or Loeffler's methylene blue followed by a final rinse in freshwater. Acid fast microorganisms appear red, whereas non-acid fast stain green or blue which are evident when examined at 1000x magnification with a brightfield microscope (Cowan 1974).

Briskly- and slow-growing isolates exist in mixed cultures, where prolific non-pathogenic species had two copies of a gene (Stahl & Urbance 1990). Research identified gene clusters types I and II. The former was encoded on the genomes of slow growing, exceedingly virulent bacteria causing swift mortality in fish and diseases in aquarists, whereas those with gene cluster II caused chronic and comparatively mild pathologies in poikilotherms, whose temperature is governed by their immediate surroundings (van der Sar et al. 2004).

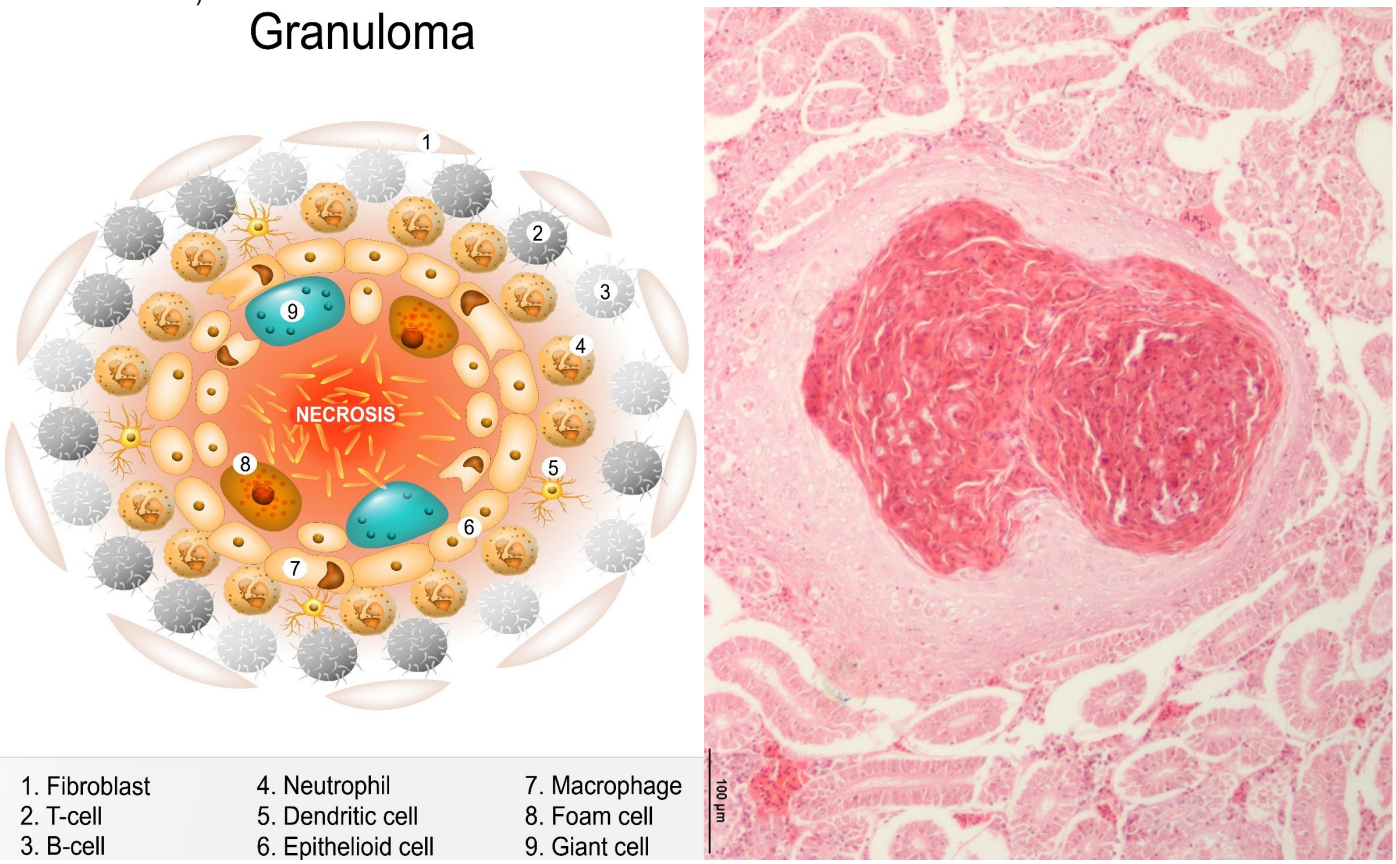

Fig 13. Left - a labelled illustration of the kind of granuloma formed by *Mycobacterium tuberculosis* and human immunity. Right - a massive visceral piscine granuloma with a necrotic centre caused by an unknown parasite. Contains public sector information licensed under the Open Government Licence (OGL) v3.0. Consult bibliography.

Remarkably, Israel's *M. marinum* are nonzoonotic (Ucko & Colorni 2005), and according to 16 svedberg (S) ribosomal ribonucleic acid (rRNA) comparative sequence analyses, isolates share over 99 percent homology with *M. ulcerans*; however, studies of multiple loci upheld them as discrete taxa (Tønjum et al. 1998). Red Sea strains contrast with the Mediterranean's, whereas globally-distributed variants remain somewhat alike (Sechi et al. 2002; Ucko et al. 2002). Nevertheless, the ancestries of bacteria whose genomes encode for pathogenicity islands, temperate phage, and plasmids cannot be delineated by comparing the sequences of a handful of genes, while extraordinarily efficacious sequence amplifications were realised with glycine-threonine-glycine (GTG)- and insertion sequence (IS)-polymerase chain reactions (PCRs; Sechi et al. 2002).

Although the formulations of agars continue to evolve, they do not remain molten at 25°C which limits us to inoculation and spreading upon solidified layers, or aseptically daily-aired thin layers of sterile broths can be incubated with a sizeable volume of air. Several millilitres are cultured in loosely-capped litre bottles lying on their side, whereas all kinds of media require supplementing with 2 percent sodium chloride (NaCl). Hence aerobic mycobacteria will grow on oleic acid-dextrose-catalase-enriched Middlebrook

7H10 agar, or in 2 percent Löwenstein-Jensen or Dorset egg media (Stahl & Urbance 1990; Francis-Floyd 2011; Colorni & Diamant 2014). The rapid doubling of opportunistic cluster II-carrying saprophytes proficient at gaining nutrition from dead and decaying remains, contrasts with the profoundly underwhelming growth of obligate pathogens which confounds swift diagnoses (Colorni & Diamant 2014).

Inoculums are made from homogenates of spleen or kidney (Colorni & Diamant 2014) which must be extensively serially diluted to ensure plate culture yields discrete colonies, because four-week aerobic incubation from 23 to 25°C (73 to 77°F) produces barely discernible colonies that turn yellow when illuminated (Colorni & Diamant 2014). Yellowing is indicative of β-carotene which is not produced by *M. tuberculosis* (Tobin & Ramakrishnan 2008), while propagation may be marginally hastened at 30°C (86°F; Francis-Floyd 2011).

Clinical Signs

Analogous to most human infections, *M. marinum* will likely manifest as chronic, where fish will exhibit lethargy, anorexia, eroded fins, discoloured skin with scale loss, and sporadic exophthalmia (pop-eye; Fig 18.). Anticipate skin ulceration and abdominal distention but asymptomatic fish may suddenly die. Necropsy will reveal whitish grey nodules on the spleen, liver, and kidney (Pro 2003; Francis-Floyd 2011; Colorni & Diamant 2014).

Several other distinct piscine pathogens cause similar lesions, whereas costly genetic analysis is the only way of confirming a diagnosis (Francis-Floyd 2011; Colorni & Diamant 2014). PCR-based methodologies can confirm *M. marinum* within two days (Francis-Floyd 2011), whereas months may transpire before conventional agar culture and DNA probe analyses yield conclusive results (Hashish et al. 2018). The bacilli of species of *Nocardia* are routinely confused with those of marine *Mycobacterium*, but they are less acid fast and pervade tissues with branching rods. Notwithstanding, Mycobacteriaceae may instigate tubercle-less ailments but acid fast rods will be evident (Francis-Floyd 2011).

Liver, spleen, and kidney are excised at the point of death, suspended in sterile phosphate buffered saline, and securely shipped to a laboratory. The dissection should be performed by personnel trained in aseptic technique wearing suitable biosafety personal protective equipment (PPE), whilst terminally-ill fish require euthanising with the veterinary anaesthetic, tricaine methanesulphonate (Finquel; MS-222). A concentration of 300 to 400 mg l^{-1} (ppm; Wingerter 2010) will humanely kill most teleosts, but leave the fish submerged for 10 minutes after gill irrigation ceases (Neiffer & Stamper 2000; Francis-Floyd 2011). Following the administration of the anaesthetic to the aerated dosing vessel, buffer the aged seawater to an equivalent pH with sodium bicarbonate as Finquel is acidic. Put a lid over to darken and cover the fish's eyes with a wet towel during transference. A compassionate "bedside manner" and euthanasia go hand in hand. At first, MS-222 can induce excitation, inasmuch as fish become disorientated, cannot right themselves, and panic. Many thus try to launch themselves skyward. However, near-death moribund fish will likely acquiesce.

Prevalence

The literature affirms that all fish are carriers or susceptible to disease (Colorni & Diamant 2014), while *Mycobacterium pseudoshottsii* were isolated from the maricultured striped bass (*Morone saxatilis*) of Chesapeake Bay (Rhodes et al. 2005; Ranger et al. 2006; Yip et al. 2007). All the same, *Mycobacterium* discovered in the same host and waters were somewhat homologous to bacteria that cause human tuberculosis (Rhodes et al. 2005a).

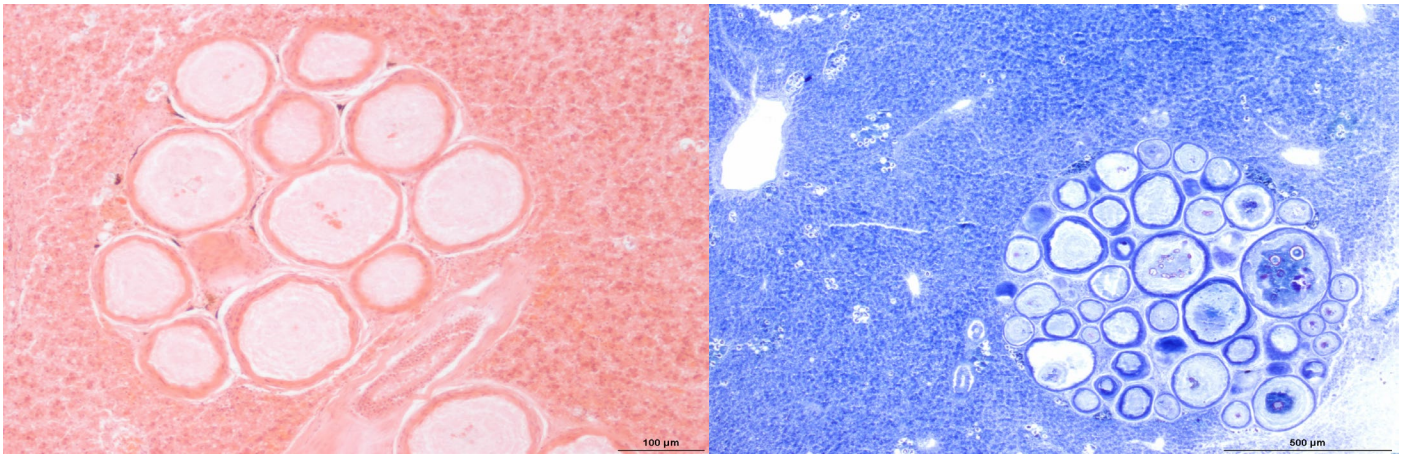

Fig 14. Multiple granulomas of the liver and kidney elicited by bacteria of the genus *Mycobacterium* in mackerel (*Scomber scombrus*). Contains public sector information licensed under the Open Government Licence (OGL) v3.0. Consult bibliography.

Increased incidences of mycobacteriosis were reported in wild and cultured teleosts in Israel's Red Sea, which were conspicuously absent in rabbitfish (marbled spinefoot; *Siganus rivulatus*) throughout the 1970s. That said, *Mycobacterium* were found in cultured seabass in 1990 where incidences escalated between 1995 and 1997 in the region's wild and cage-cultured fish. Outbreaks occurred off a beach, and on a reef, where 21 to 42 percent of rabbitfish were infected (Diamant et al. 2000).

Striped bass experimentally challenged with *Mycobacterium shottsii* and *M. gordona* developed mild to chronic splenic pathologies, where fish made full recoveries without recrudescence (Gauthier et al. 2003). Susceptibility varies, and although *M. marinum* cause significant diseases of seabass (*Dicentrarchus labrax*), turbot (*Scophthalmus maximus*), Florida pompano (*Trachinotus carolinus*), and Syngnathidae such as pipefish and seahorses, potential hosts or reservoirs include oysters, mice, opossums, bats, reptiles, amphibians, manatees, sea lions, dolphins, and small-toothed whales (Clark & Shepard 1963; Beecham et al. 1991; Francis-Floyd 2011).

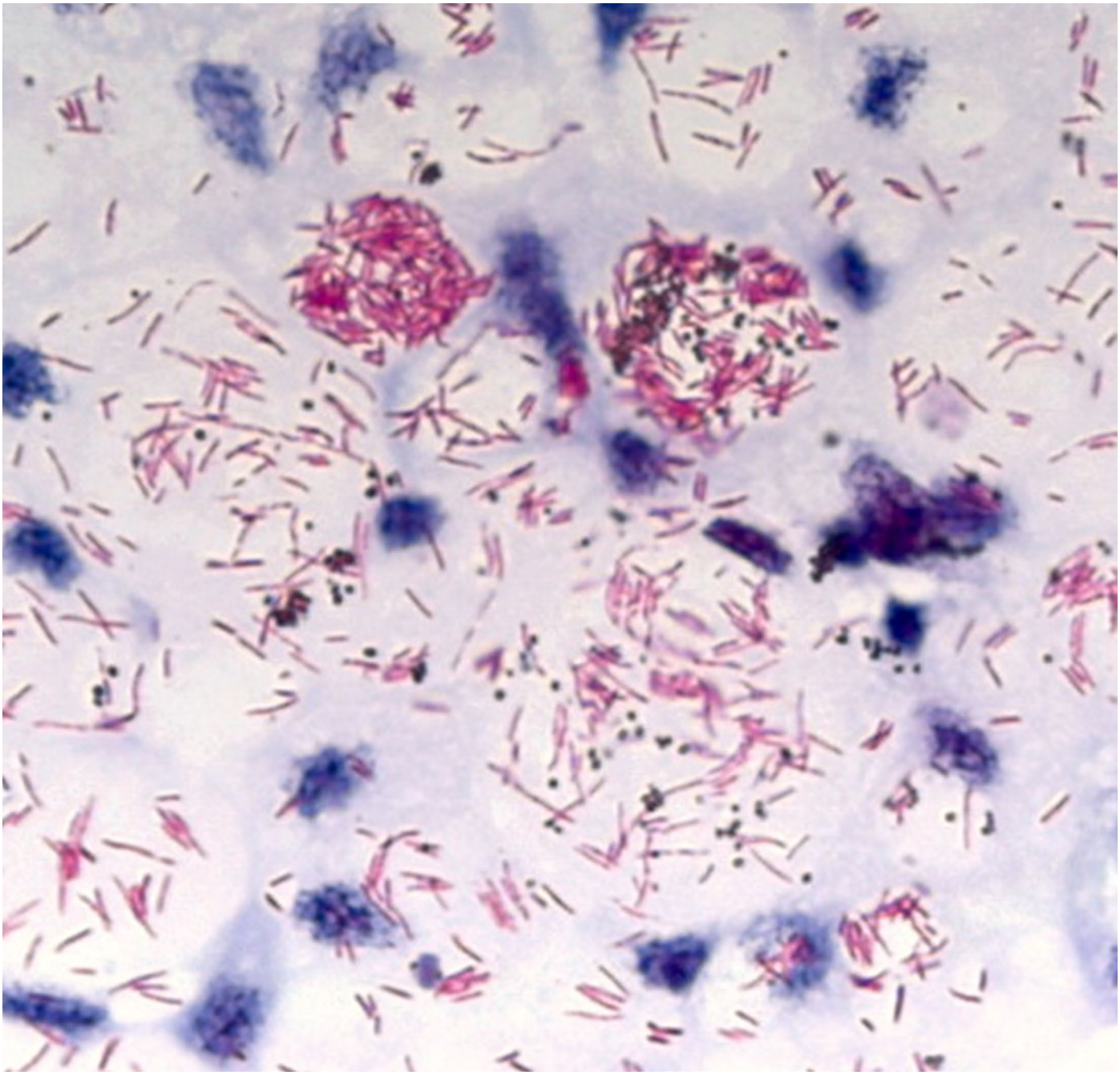

Fig 15. An acid fast stain of *Mycobacterium* bacilli from a tropical freshwater fish at 1000x magnification. Courtesy of https://FishPathogens.net

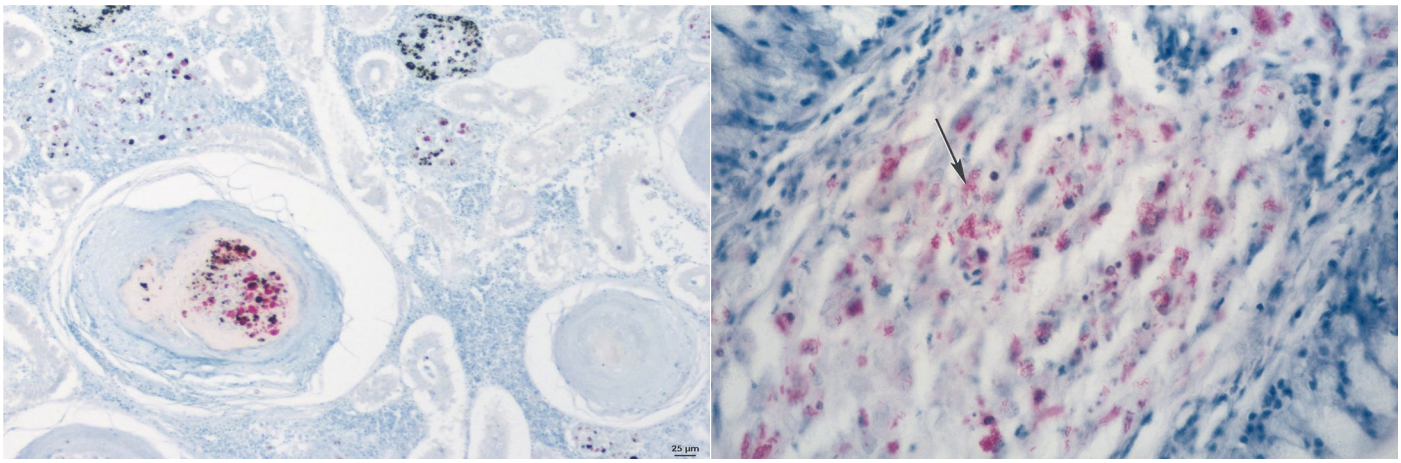

Fig 16. Acid fast stains of unencapsulated granulomas comprising species of *Mycobacterium* in mackerel. Contains public sector information licensed under the Open Government Licence (OGL) v3.0. Consult bibliography.

Cultured striped bass intended to bolster wild populations in northern California's Sacramento and San Joaquin estuaries, were ravaged by a virulent strain of *M. marinum* in the 1980s. 900 yearlings died, whilst 80 percent of the remainder became ill (Hedrick et al. 1987). *Mycobacterium marinum* and *Mycobacteroides chelonae* were isolated from Portugal's cultured turbot, where pathology was observed in merely 2 percent of fish, but both microbes were present in all (dos Santos et al. 2003). Gut-bound species of

Mycobacterium were discovered in 16.5 percent of aquarium-maintained Atlantic horse mackerel (*Trachurus trachurus*), whilst the study concluded intestinal incursion was the principal route of contagion (Ortega et al. 2014).

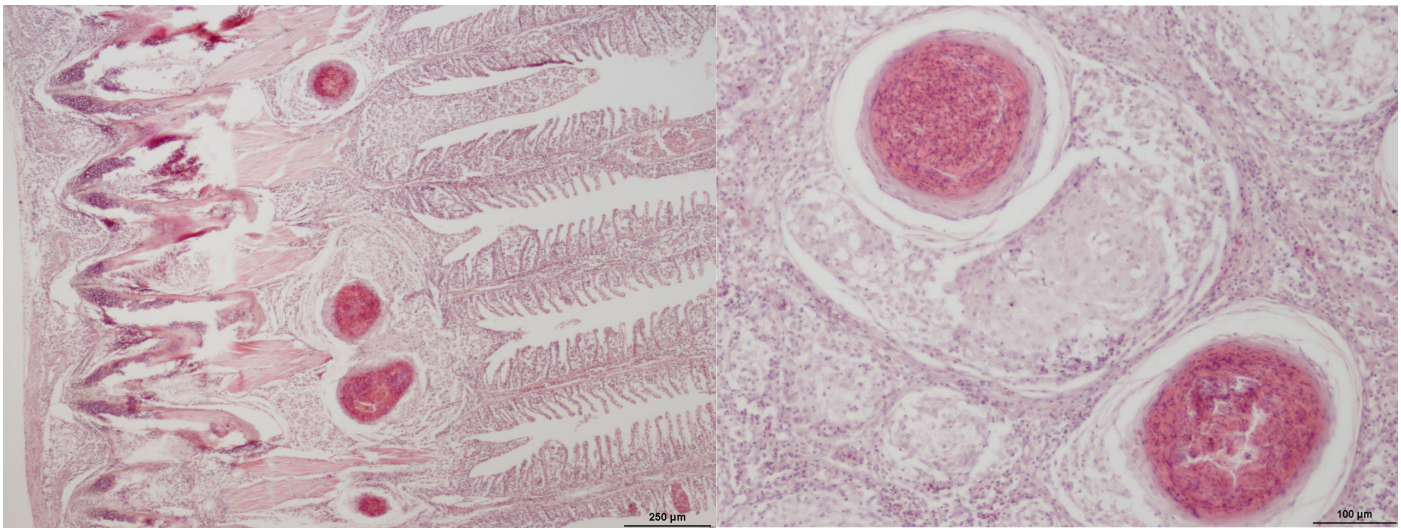

Fig 17. Necrotic granulomas of gill connective tissue sampled from a freshwater cichlid. Putative pathogen, an acid fast strain of the genus *Mycobacterium* or *Nocardia*. Contains public sector information licensed under the Open Government Licence (OGL) v3.0. Consult bibliography.

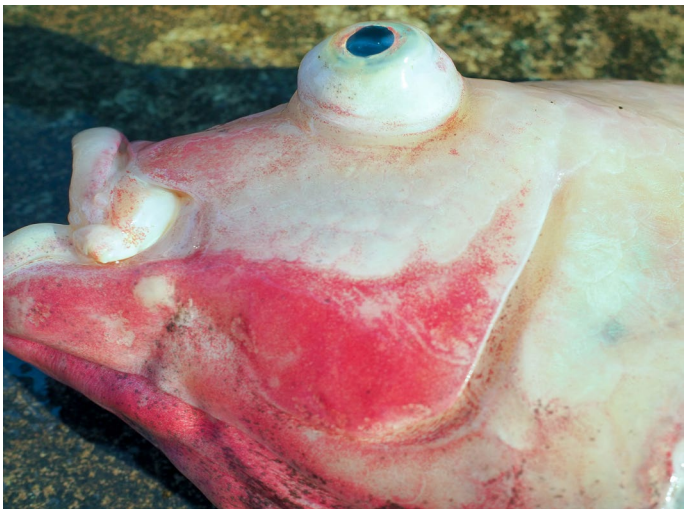

Fig 18. Bacterial "pop-eye" (exophthalmia), inflammation, and petechial haemorrhages.

M. marinum infected northwestern Italy's cultured hybrid striped bass in 2008 (Bozzetta et al. 2010).

Virulence Factors

M. marinum with cluster I typically inhabit the immunity-conferring leukocytes of their host (Knobloch et al. 2020) where the 60- to 90-carbon-long hydroxylated lipid tails of the bacterial envelope called mycolic acids, appear vital for intracellular survival (Marrakchi et al. 2014; Hashish et al. 2018). An extraordinary variety of complex cell wall-integral fatty acids contribute to virulence (Brennan 2003), where some moderate the manufacture and liberation of cytokines from leukocytes, and nullify the cellular (cyto)toxicity of TNF-α-induced macrophages (Reed et al. 2004).

Poor prognoses are associated with elevated temperatures and diminished dissolved oxygen (DO) in hypoxic waters (Jacobs et al. 2009) where like all prokaryotes, inorganic dissolved iron (Fe) remains essential for mere survival and virulence. *Mycobacterium marinum* liberate Fe^{3+}-scavenging siderophores known as mycobactins which are later internalised to release their cargo. Macrophages and phagocytes phagocytose these microbes within phagosomes with the intention of destroying them, but they multiply, avoid intraphagosomal-killing, and gain access to the host's intracellular liquor known as the cytosol (Fig 3.; Knobloch et al. 2020).

Macrophages engulf pernicious variants of *M. marinum* at rates 40 to 60 times greater than their innocuous counterparts (Barker et al. 1997; El-Etr et al. 2001), while their virulence determinants inhibit vesicle and lyso-somal fusion responsible for phagosomal destruction (Barker et al. 1997). The survival of profoundly pathogenic gene cluster I-encoding *M. marinum* in human and carp leukocytes far exceeds that of species and strains whose genomes encode for cluster II (van der Sar et al. 2004), wherein they manipulate the activities of host macrophages and initiate granulomas with innate defence (Davis et al. 2003).

These microbes adapt the intracellular scaffolds of the stricken by stimulating the polymerisation of actin which unbeknown to immunity, covertly transports them into adjacent cells (Fig 3.; Stamm et al. 2003). Macrophages initially limit microbial dissemination but are ultimately responsible for dispersal throughout mid- to late-contagion. However, intracellular development is curtailed following the induction of adaptive immunity (Figs 21. to 24.; Clay et al. 2007).

Some mycobacterial gene products include repetitive residues of the amino acid motifs, proline-glutamine (Pro-Glu; PE) or proline-proline-glutamine (PPE), which are necessary for intra-macrophage and -granuloma persistence (Daleke et al. 2011), yet molecular peculiarities such as these have proven efficacious vaccines. For instance, unmethylated CpG motifs are single stranded (ss)DNA molecules encoding consecutive cytosines and guanines (Weiner et al. 1997), albeit absent in vertebrate genomes, they remain abundant in bacterial (Kuramoto et al. 1992). These powerful immune modulators elicit robust inflammatory responses and stimulate the liberation of B- and natural killer (T_k)-cell-inducing cytokines (Fig 23.; Krieg 2002).

Affiliates of the mmaA family of microbial enzymes bond cyclopropane to bacterial fatty acids which confers a profound resistance to the reactive oxygen species (ROS) of macrophages called oxidative bursts, whereas the mycobacterial enzyme L-carnitine dehydratase digests the intracellular L-carnitine of their host whose breakdown liberates vital inorganic carbon (C) and nitrogen (N; Tønjum et al. 1998).

The polyketide synthase family of genes encode proteins that expedite the biosynthesis of the fatty acid tails of bacterial cell membranes, while others remain necessary for the manufacture of mycobacterial amino and nucleic acids. The sulphur-containing amino acid cysteine which remains vital for the stabilising intramolecular disulphide bridges of globular proteins, appears in short supply in macrophages. However, the *cys* operon of mycobacteria comprises *cysD* and *cysQ* so they can produce their own (Ruley et al. 2004), whereas AraC encoded on *Rv1931c* confers an aptitude for utilising the C- and N-comprising L-arabinose of their host. Hence the expression of *cysD*, *cysQ*, and *Rv1931c* promotes intramacrophage proliferation and survival (Frota et al. 2004; Ruley et al. 2004; Hashish et al. 2018).

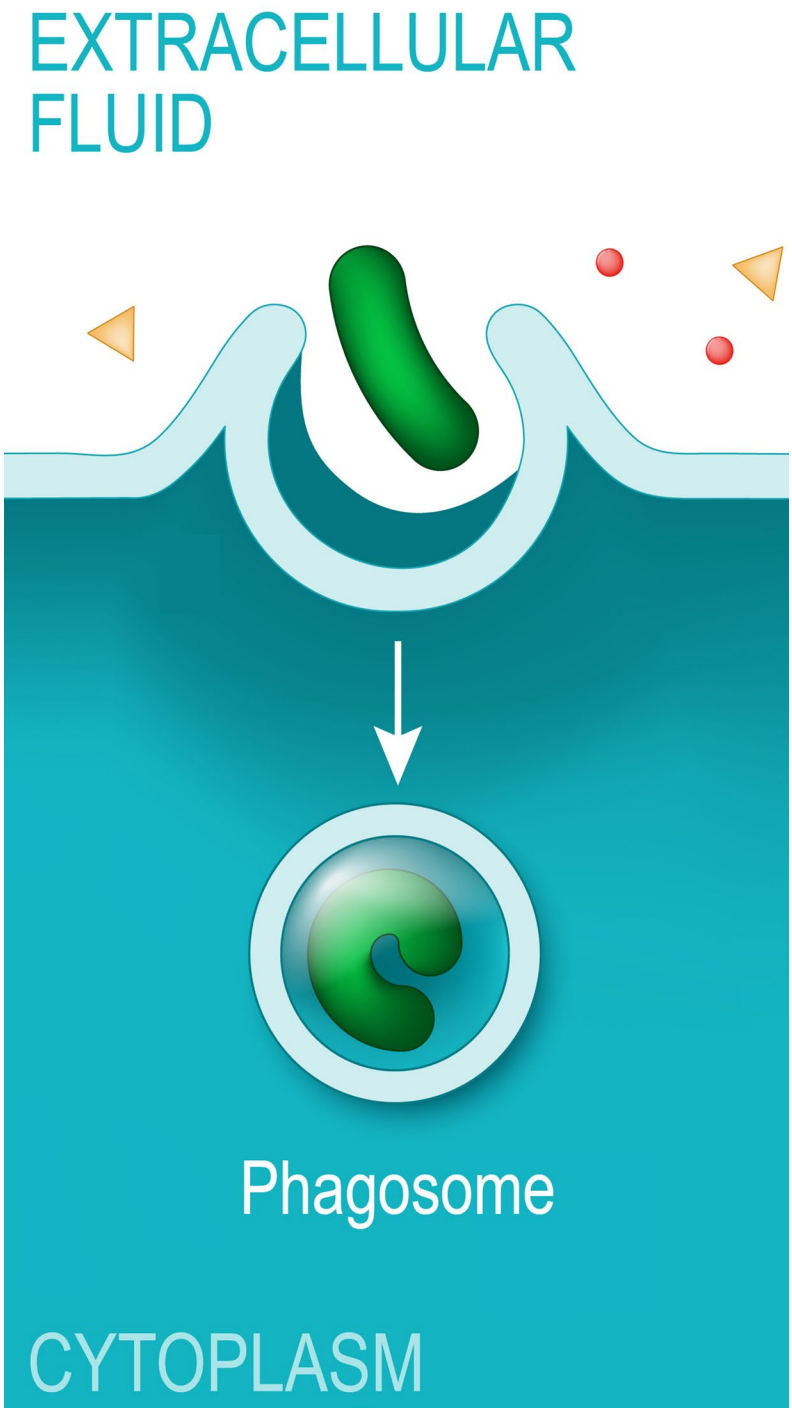

Fig 19. Phagocytosis or cell eating whereby pseudopodia envelop foreign bodies or debris and internalise them in phagocytic vesicles, which later fuse with enzyme-comprising others called lysosomes which normally digests incarcerated pathogens.

ESX-1 or the type VII secretion system (T7SS) encoded on the mycobacterial *esx-1* cluster is merely one of five delivery devices used by these microbes to export virulence determinants (Sassetti & Rubin 2003, cited in Hashish et al. 2018; Abdallah et al. 2009). ESX-1s, their auxiliary proteins, and the extracellular products they disseminate, prove indispensable for adapting to diverse hosts (Sassetti & Rubin 2003, cited in Hashish et al. 2018; McLaughlin et al. 2007; Weerdenburg et al. 2015; Hashish et al. 2018). ESX-1s primarily excrete the key virulence effectors ESAT-6s (McLaughlin et al. 2007; Coros et al. 2008; Osman et al. 2022), where homologues of the T7SS substrate gene, *esxAB* are carried by *M. tuberculosis* and *M. marinum* which retains two copies. Expression of *esxAB* appears vital for intracellular infections of laboratory-maintained carp leukocytes and may thus influence host range (Weerdenburg et al. 2015).

The *espL* locus of the *esx-1* gene cluster seems a prerequisite for tubercle formation, virulence, and the secretion of the ESX-1 substrates: ESAT-6 and EspE (Stoop et al. 2011), whilst the gene products EsxA and EsxB remain key membrane components of T7SS (Kennedy et al. 2014).

A study discovered polypeptides with repetitive arrays of 100 to 180 residues of proline-glutamine (PE) or proline-proline-glutamine (PPE; Daleke et al. 2011). The genomes of benign saprophytes like *Mycobacterium smegmatis* encode few PE and PPE proteins, yet they are synthesised in great numbers by pathogenic species and strains. They are therefore considered virulence determinants many of which are expressed on cell surfaces (Abdallah et al. 2006; Abdallah et al. 2009).

Mycobacteria distribute PPE41 via the ESX-5 secretion system encoded on the gene cluster *esx-5* (Abdallah et al. 2006; Abdullah et al. 2009). Triacylglycerol lipases (LipYs) are synthesised with signal sequences in benign rapidly-growing Mycobacteriaceae like *Mycolicibacterium gilvum*, yet those of *M. tuberculosis* and *M. marinum* are conjugated to a PE or PPE domain that appear necessary for exportation. These motifs do not become part of the functional protein and are subsequently cleaved (Daleke et al. 2011). Mutants lacking ESX-5$^-$ were more virulent to fish than wildtype but were less able to survive in their embryos. It seems therefore, that this secretion system assists the establishment of steadfast non-overwhelming infections (Weerdenburg et al. 2012). Host demise results in parasite mortality, so every pathogen's intention is to instigate merely moderate discomfort and malaise. Hence given sufficient time, an equilibrium is attained between the sufferer and its parasites.

M. marinum's two gene clusters encoding SecA proteins: *SecA1* and *SecA2*, differentiate them from other Mycobacteriaceae. The products of SecA1's genes undertake housekeeping (HK), whereas the roles of SecA2's are less clear but appear to facilitate excretion. *SecA2*$^-$ mutants were less virulent and unable to emit the antioxidant enzyme, superoxide dismutase (SOD) that confers resistance to the lethal oxidative bursts of macrophages and other "white cells" by reducing ROS like hydrogen peroxide (H_2O_2;

Braunstein et al. 2003). However, later research established this cluster's proteins are necessary for energy-generating membrane-bound ATPases, components that enhance the integrity of the bacterial envelope, and the non-PE or -PPE-chaperoned secretion of extracellular products that assist tubercle formation and modulate host defence (Braunstein et al. 2003; Watkins et al. 2012). Effectors exported by SecA2-type proteins inhibit the fusion of phago- and lyso-somes which abets macrophage occupancy (Sullivan et al. 2012), whereas SecA2s localise the phosphorylating enzyme and virulence determinant, protein kinase G (PknG) in the bacterial envelope. The role of SecA2 was thereafter designated the accessory translocation pathway (Woude et al. 2014).

Fig 20. A watchman goby (*Cryptocentrus cinctus*) presenting with an erosion-like ulceration surrounded by a white border, likely colonised by chromists, fungi, and bacteria, yet these kinds of lesions are commonly initiated by the latter two. Courtesy of Liam Furlong ©, Staffordshire, UK.

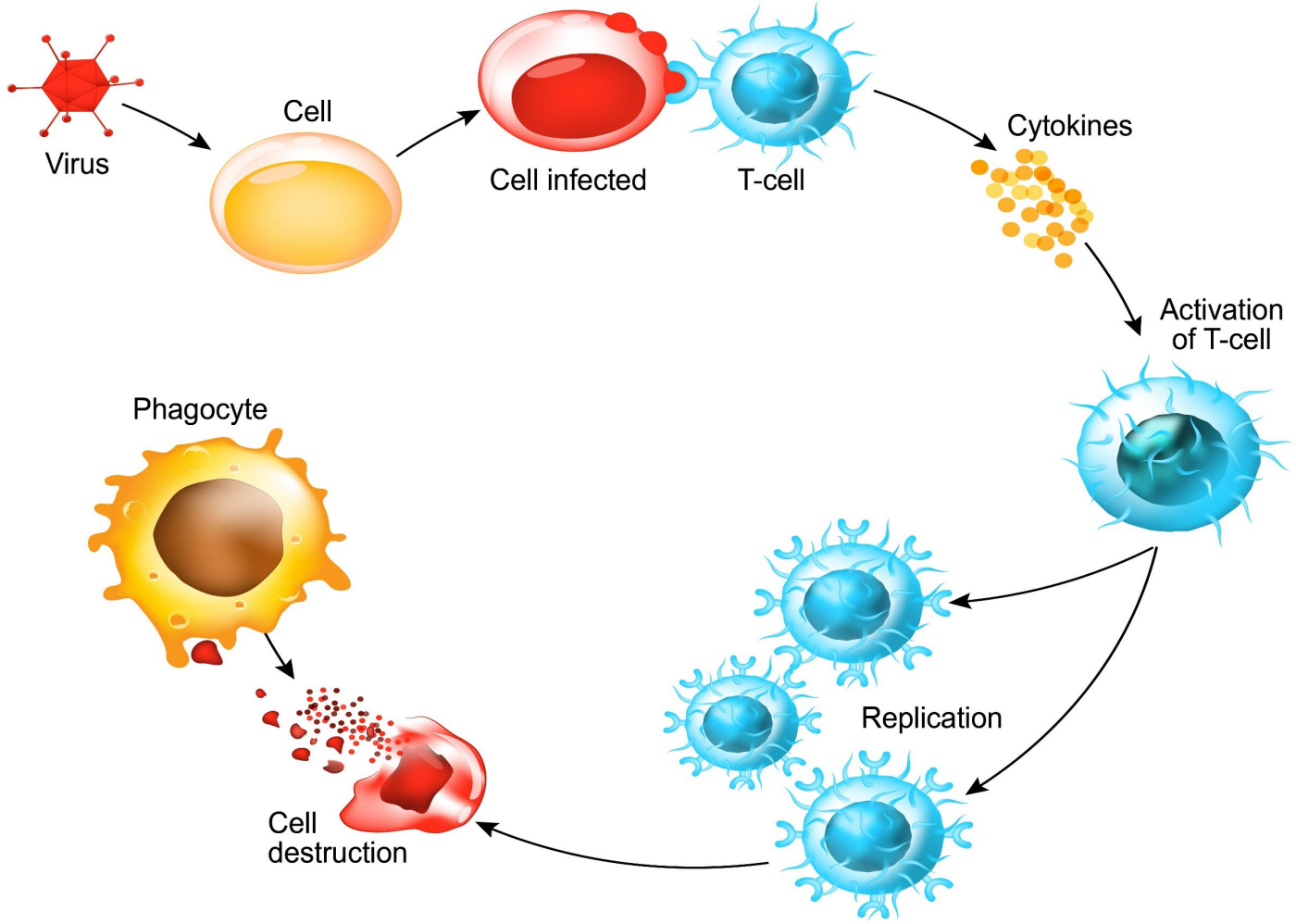

Fig 21. T-cell activation, replication, and ablation of an intracellular pathogen or a cell with pronounced genetic insults.

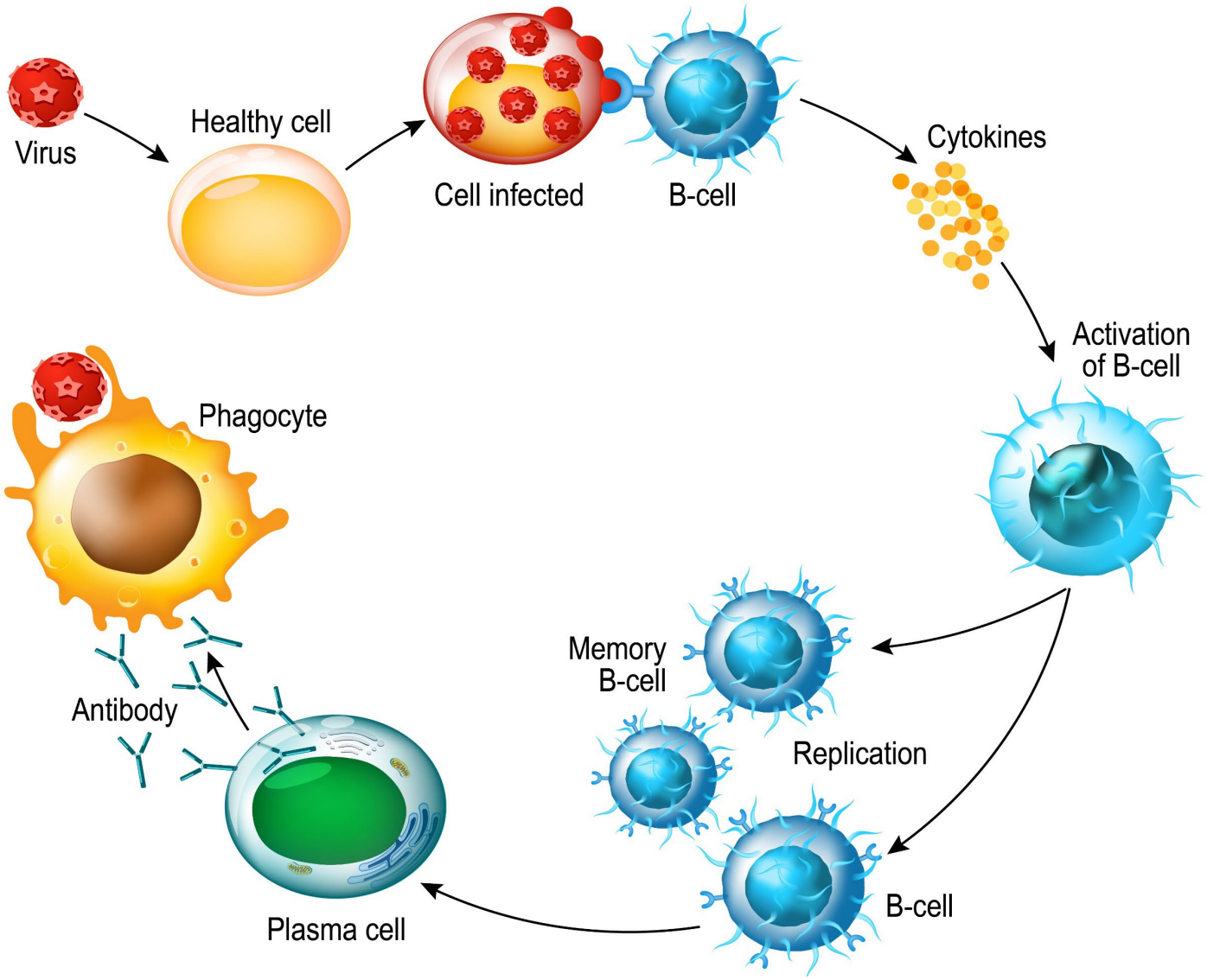

Fig 22. B-cell activation, antibody production, and phagocytic engulfment of a virus-, intracellular pathogen-infected, or aberrant cell.

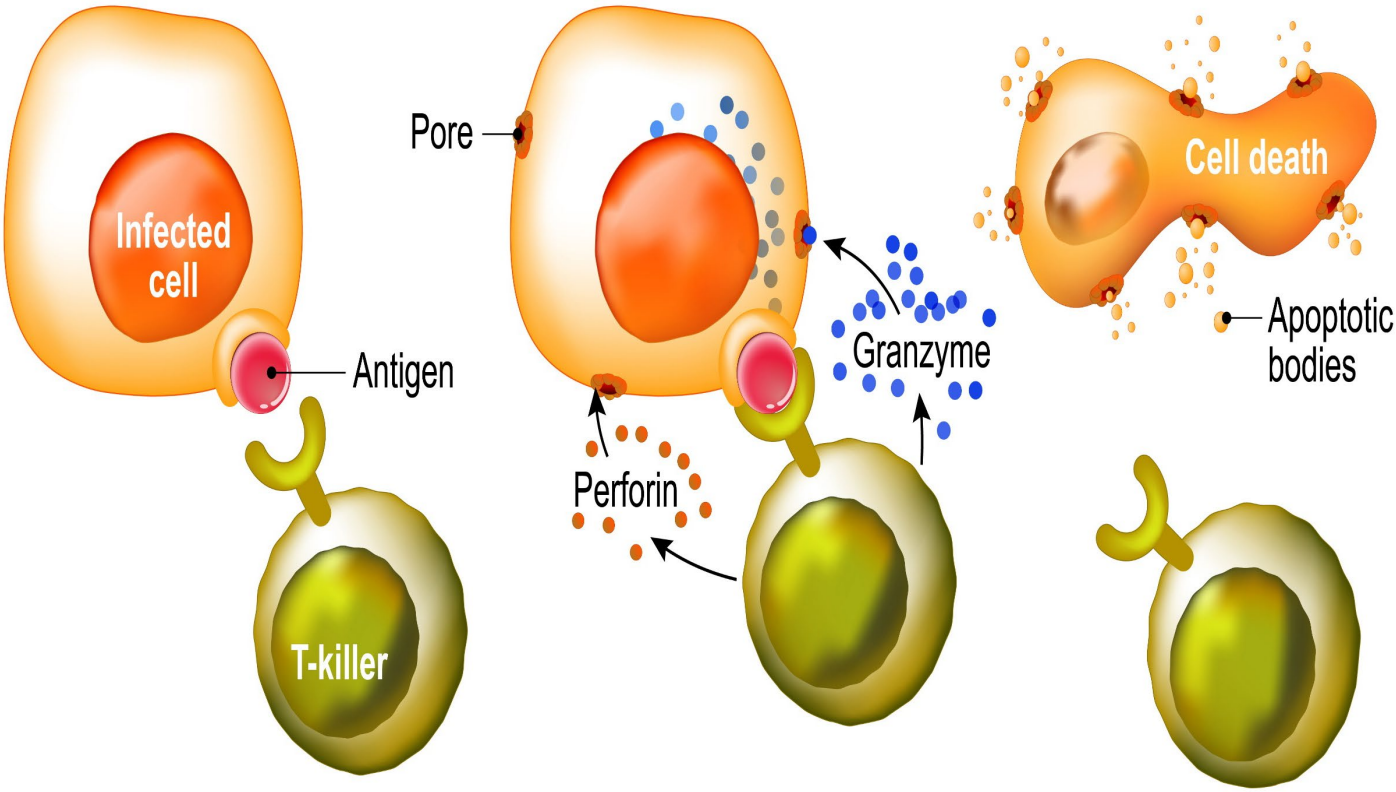

Fig 23. The receptor of a finfish CD8 killer-like T-lymphocyte binds the “foreign” peptide presented by a major histocompatibility (MHC) class I molecule of an infected host cell, where activation releases apoptosis-inducing perforin and granzyme.

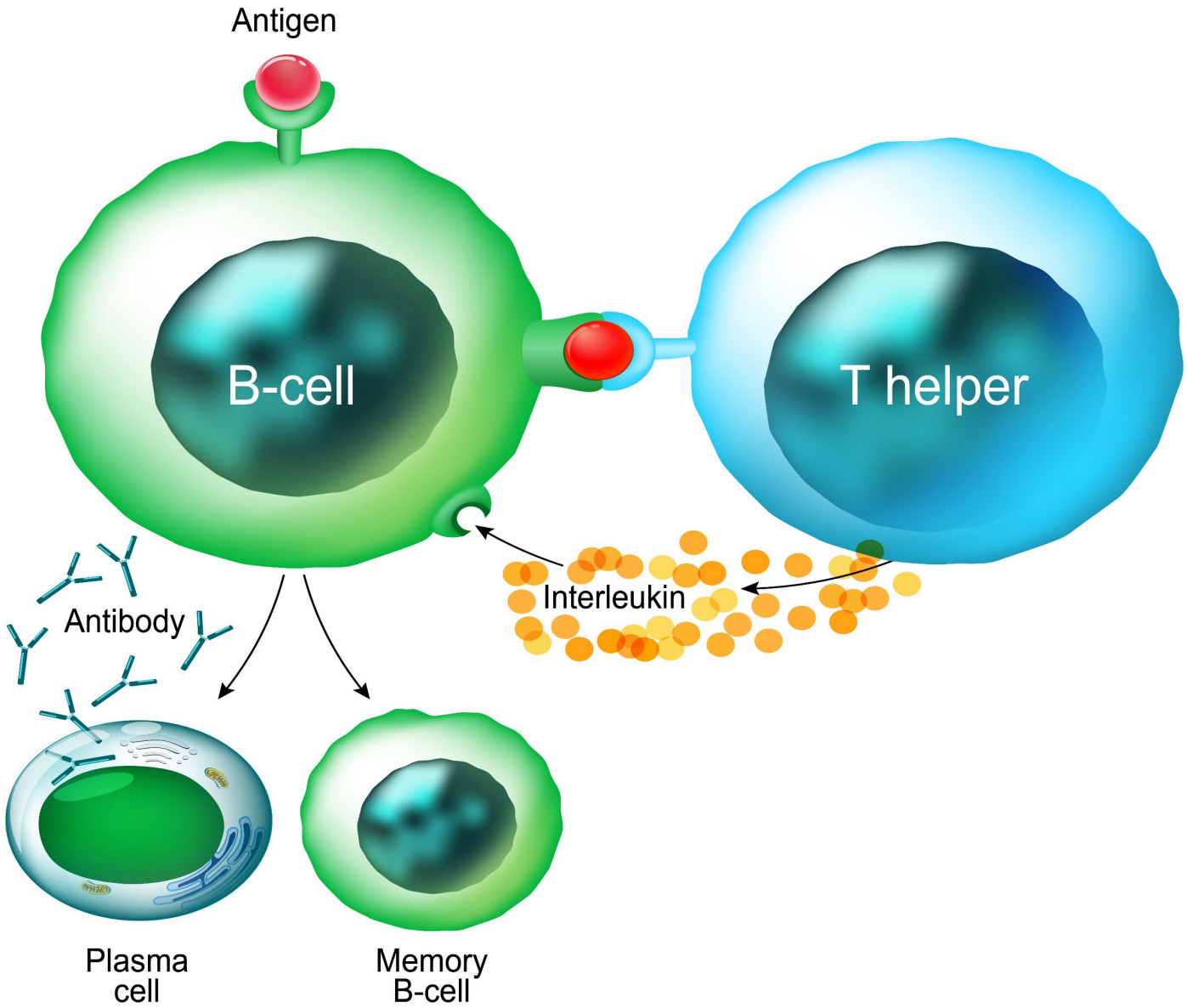

Fig 24. T-helper recognition, signalling, and the production of B-memory-retaining and antibody-producing plasma cells. However, piscine long-lived plasma cells also assume the role previously assigned to memory cells (Rijkers et al. 1981; Wu et al. 2019).

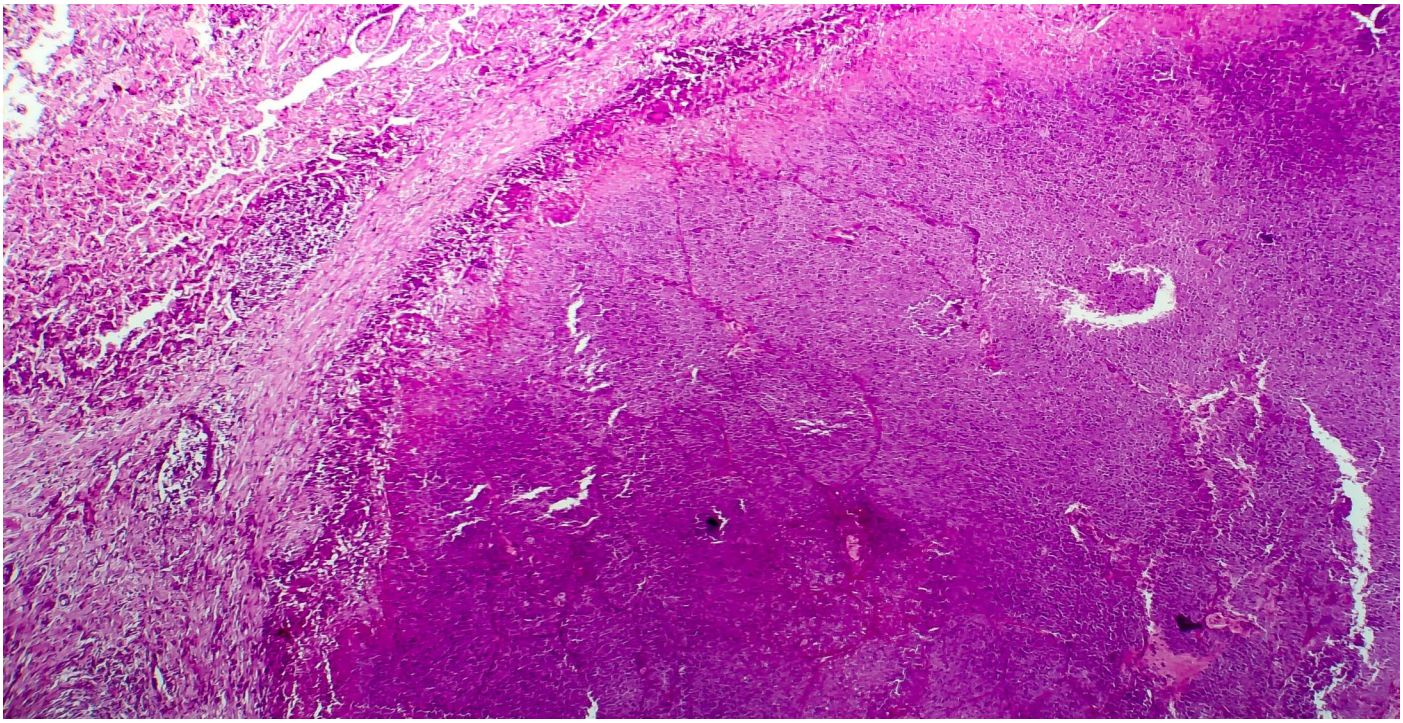

Fig 25. A micrograph of a section of the outer circumference and contents of a *Mycobacterium* granuloma stained with haematoxylin and eosin (H & E).

Phenolic glycolipids (PGLs) and phthiocerol dimycocerosates (PDIMs) are outer envelope constituents and critical virulence effectors that interfere with cell-to-cell communication and manipulate eosinophils in such a way as to curb the destruction of their internalised microbes (Fig 5.; Sassetti & Rubin 2003, cited in Hashish et al. 2018; Huang et al. 2017).

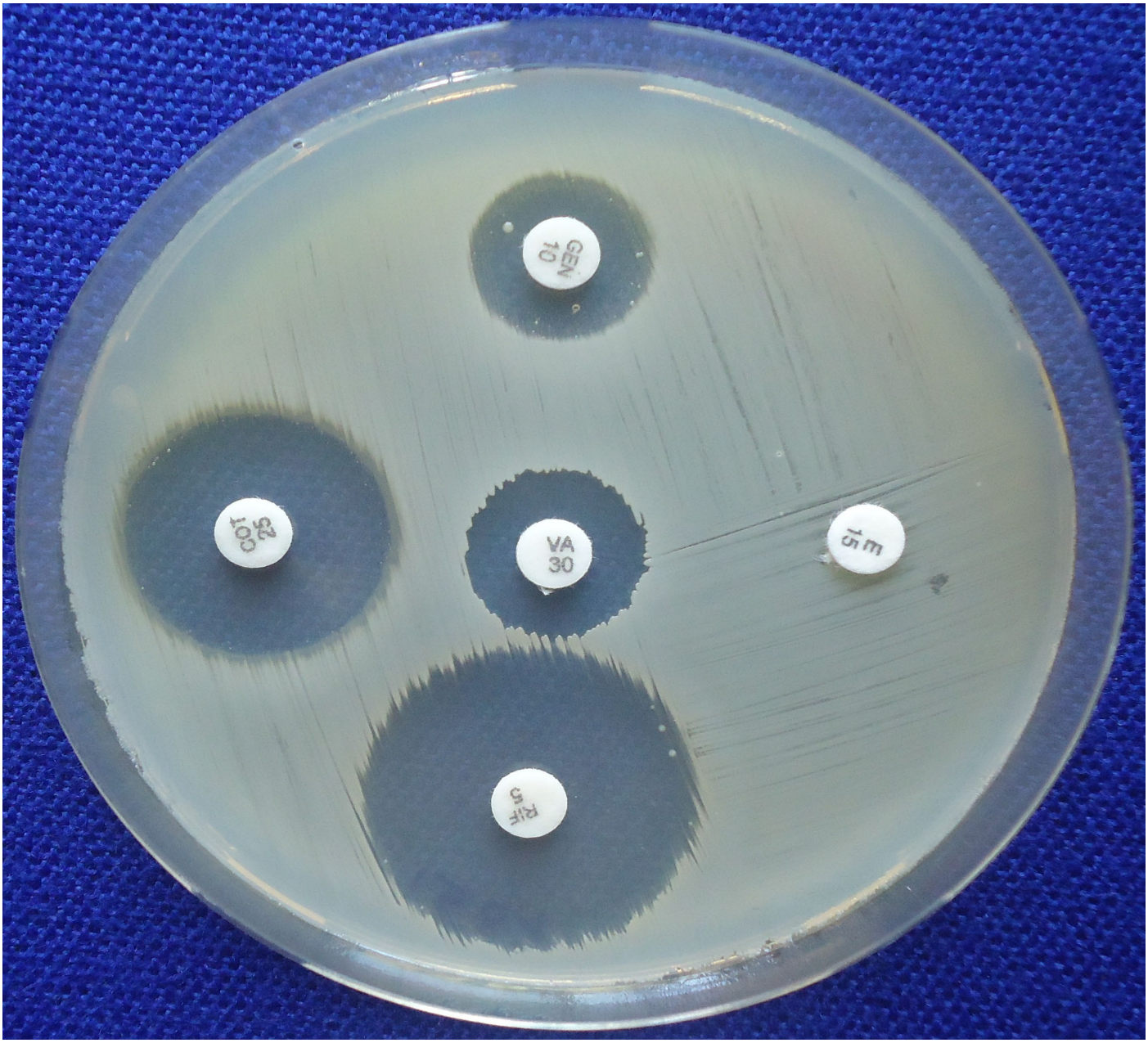

Fig 26. Screening for antibiotic sensitivity on solidified NaCl-enriched nutrient agar inoculated with a lawn of biofilter microbes and mini Whatman filter papers impregnated with antimicrobials. Clearings indicate sensitivity, while the consortium is resistant to E15. Thorough appraisals, however, are conducted in triplicate and include a nonimpregnated sterile blank that eliminates autogenous toxicity.

The *mce* gene cluster consists of four operons: *mce 1* to *mce 4* under the control of a single promoter which are thus transcribed together. *mce 1*$^{-}$ mutants are more pathogenic and contagious than wildtype, whereas the products of *mce 3* and *mce 4* enhance intracellular survival and persistence, yet their functions are very different to those of MCE 1 (Riley 2008; Sáenz-Lahoya et al. 2019). Macrophages activated with gamma-interferon (γ-INF) sequester bacteria in carbon-limited phagosomes with membranes that comprise cholesterol. *mce 4* encodes for machinery that imports and degrades cholesterol which guarantees these bacteria a life-sustaining source of C (Pandey & Sassetti 2008).

The novel cytosine to thymine base substitution at position 395 in *MMAR_0911* (*grcC1*) leads to an alanine to valine substitution at residue 132 of the enzyme polyprenyl diphosphate synthase implicated in the biosynthesis of menaquinones and components of the bacterial cell wall. This natural mutation equips an extensive variety of *Mycobacterium* with low-level resistance to the antimicrobial linezolid and the antibiotics clarithromycin, cefoxitin, clofazimine, levofloxacin, moxifloxacin, rifampicin, and vancomycin, by feasibly reducing cell wall permeability. This makes GrcC1 a potential target for novel chemotherapeutics as its absence increases susceptibility to linezolid and the aforementioned antibiotics (Fang et al. 2025).

Control/Prevention

Substandard water quality and poor nutrition contribute to disease (Francis-Floyd 2011) while species of *Mycobacterium* are ubiquitous which implies optimised systems and excellent husbandry are sufficient to sustain subclinical infections (Pro 2003). All the same, virulence and immunity are likely regionalised, and thus mixed-origin hosts are bound to respond very differently. 2 percent

of the brewer's yeast-based immunostimulant GroBiotic®-A protected fish from *Mycobacterium marinum* (Li & Gatlin 2005).

Corals and fish voraciously consume the human food supplement brewer's yeast which is a powerful immunostimulant that comprises RNA, vitamins, and minerals including a beneficial rare biologically-active form of chromium (Scawarz & Mertz 1959; Ferreira et al. 2010), where feeds may contain up to 50 percent (Oliva-Teles & Goncalves 2001). However, when fed regularly in reefs, its soluble ingredients are likely analogous to potentially hazardous carbon dosing (Kline et al. 2006; Feldman et al. 2011), so use as an occasional treat.

The antibiotics erythromycin, kanamycin, minocycline, rifampicin, and streptomycin are somewhat efficacious. However, dietary administration is required for several months which often proves unsatisfactory, while therapies must be administered in adequately-mature and antibiotic-resistant quarantine (Pro 2003) insofar as most antibiotics are lethal to biofilter consortia (Fig 26.).

Conjugable DNA plasmids that are easily transferred from one bacterium to another, often confer antibiotic resistance which are extensively distributed amongst biofilter microbes (Watts et al. 2017). Erythromycin, ethambutol, clarithromycin, isoniazid, rifampicin, and streptomycin alleviate symptoms but create carriers (Colorni et al. 1998, cited in Colorni & Diamant 2014), whereas eight-months of a combination of rifampicin, doxycycline, and enrofloxacin successfully treated freshwater teleosts (Strike et al. 2017).

Aquatic mycobacteria do not ordinarily infect humans because temperatures above 30°C tend to be nonpermissive (Ranger et al. 2006) and thus raising the temperature of a *fish-only* system to 30.5°C may prove efficacious. However, gradual increments are mandatory, and the system must be optimally designed and maintained.

Mycobacterium ulcerans carry transmissible plasmids that encode macrolide toxins which grant survival at elevated temperatures. Plasmid-infected *M. marinum* therefore grow above 30°C which can instigate deep ulcerations or internal systemic infections in humans (Ranger et al. 2006). There have been reports of purple tubercles leading from foci of infections, arthritic-like symptoms originating from deep punctures, or fatal systemic pathologies of immunocompromised patients (Ang et al. 2000; Pro 2003; Hashish et al. 2018).

Intraperitoneal (IP) injections of streptomycin or the juice squeezed from a clove of garlic (allicin; *Allium sativum*), mitigate mycobacteriosis in seabass, yet partial recouperation was observed in the unimmunised control group. All fish were actively expressing antibodies to *M. marinum* before the challenges, which suggests persistent exposure is unprotective, whereas antibiotic cessation caused upsurges of antibodies because streptomycin is immunosuppressive (Colorni et al. 1998). IP injections should only be performed by veterinary surgeons or suitably qualified livestock managers.

Intriguingly, recombinant cow *Mycobacterium bovis* BCG with deletions in their ESX-1 secretion systems were used to vaccinate Japanese flounder (*Paralichthys olivaceus*) and amberjack (*Seriola dumerili*) which induced lasting protective immunity (Kato et al. 2011; Stoop et al. 2011).

A recent study investigated the PE/PPE family of *Mycobacterium* proteins whose MMAR_1296 brought about alterations in cellular architecture and increased the likelihood of destruction in harsh environments like the phagosomes of macrophages, when the protein was expressed by a recombinant strain of saprophytic *Mycobacterium smegmatis.* These bacteria upregulated immune pathways in experimentally-inoculated mice, which conferred significant resistance to subsequent challenges with *Mycobacterium abscessus* and *Mycobacterium marinum*. MMAR_1296 may thus prove a useful vaccine (Xie et al. 2025).

Although the scientific community has made vital inroads, species of *Mycobacterium* will persist in facilities and aquaria for several years and will be virtually impossible to eradicate (Francis-Floyd 2011; Colorni & Diamant 2014). Despite the author evading infection over the last five and a half decades, do not expose cuts or grazes to system water and exercise caution around venomous or pugnacious residents.

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A marine aquarium, reef tank, and saltwater fish system guide, handcrafted by a coral reef expert.

Chris Aslett

As the principal director of Reef Ranch Publishing Ltd (https://reefranch.co.uk/) and author of The Complete Reef Aquarist – A Conservation Manual (https://reefranch.co.uk/reef-aquarium-guide/), Chris has over 55 years of experience keeping aquatic animals with 47 of them nurturing marine species. His innate passion for system dynamics drove him from the laboratory to university where he gained a greater appreciation of biochemistry, biotechnology, epidemiology, genetics, histology, inorganic and organic chemistry, mariculture, molecular and microbiology, saltwater zoology, and the diagnosis and treatment of aquatic diseases. His dedicated marine livestock supplier, The Reef Ranch™, demanded he devise, streamline, and establish protocols for combined acclimation and prophylactic pest/parasite clearance, and innovate system design, optimisation, maintenance, and husbandry in the face of incessant influxes of hundreds of delicate marine animals. With exceptional, uncompromising, and likely the UK's most disease-free reef and fish-only facilities, losses were less than one resident every six months. 20 years hence, he has refined his expertise for digesting, authoring, editing, and publishing reef conservation-driven scientific literature (https://reefranch.co.uk/reef-research-and-aquarium-advice/), to an end of diminishing the impact the tropical marine ornamental industry exerts in the wild.