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SEPTIC THROMBOPHLEBITIS AND EMBOLIC COMPLICATIONS IN AN 18-YEAR-OLD MALE: A RARE CASE OF REVERSE LEMIERRE'S SYNDROME

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ABSTRACT

Reverse Lemierre's syndrome (RLS) is a rare and potentially life-threatening condition defined by septic thrombophlebitis arising from an extra-oropharyngeal source, often leading to the development of septic emboli. We present the case of an 18-year-old male who developed RLS following right thigh pyomyositis precipitated by a minor muscular strain. This case illustrates the clinical features, diagnostic steps, and treatment decisions required for managing RLS. Making the diagnosis promptly is vital, and a combination of tools, including ultrasound, MRI, and CT pulmonary angiography, can help pinpoint the condition early, allowing for timely and effective intervention. In this patient, a multidisciplinary approach combining targeted antibiotics, anticoagulation, and surgical drainage led to a successful recovery. This case highlights the need to consider uncommon sources of sepsis in young patients, as early recognition and timely intervention can greatly improve their outcomes.

KEYWORDS: *Reverse Lemierre's syndrome, pyomyositis, septic pulmonary embolism, deep vein thrombosis, Staphylococcus aureus*

INTRODUCTION

Lemierre's syndrome, also known as post-anginal septicemia or necrobacillosis, was first reported in 1890 by Courmont and Cade. However, it was more comprehensively described in 1936 by André Lemierre, a French physician and professor of microbiology, in a review of 20 cases published in the *Lancet* (1). Lemierre's syndrome refers to thrombophlebitis of the internal jugular vein accompanied by bacteremia, typically following an oropharyngeal infection and most often caused by anaerobic bacteria. When a similar thrombotic and infectious process occurs in the lower limb, it is termed reverse Lemierre's syndrome (2). *Fusobacterium necrophorum* is a gram-negative anaerobe that can cause localized throat infections or severe systemic illness. Systemic involvement is known as Lemierre's syndrome, post-anginal sepsis, or necrobacillosis (3).

Pyomyositis is a primary bacterial infection of the skeletal muscles, commonly referred to as tropical myositis due to its higher prevalence in individuals residing in tropical regions. The condition is often diagnosed late, leading to increased morbidity and, in some cases, significant mortality (4). *Staphylococcus* species, particularly *Staphylococcus aureus*, are the most common causative organisms in pyomyositis, especially when the lower limbs are involved, accounting for

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approximately 90% of cases (5). Unlike classical Lemierre's syndrome, reverse Lemierre's syndrome often lacks ENT symptoms, making early diagnosis difficult. This case describes a healthy young male who presented with fever and limb pain, later found to have thigh pyomyositis, femoral vein thrombosis, and septic pulmonary emboli. It highlights the need for early imaging and clinical suspicion in atypical sepsis presentations.

CLINICAL PRESENTATION

An 18-year-old previously healthy male presented with high-grade fever and painful swelling of the right thigh for three days. He reported a history of thigh strain sustained while carrying a heavy water jar downstairs. The next day, he developed worsening pain and diffuse swelling in the right thigh, restricting movement. Fever with chills followed, partially relieved by over-the-counter medication. He was initially evaluated at a local hospital for sepsis with polyarthralgia and referred to our center for further management.

At admission, he was febrile (103°F), conscious, and oriented. Vitals included pulse 104 bpm, blood pressure 118/80 mmHg, respiratory rate 24/min, and oxygen saturation 94% on room air. Bilateral rales were heard on chest auscultation. Local examination revealed diffuse swelling, warmth, and tenderness in the right thigh without rash or lymphadenopathy (Fig.1).



Fig. 1. Right thigh swelling- vastus lateralis pyomyositis.

INVESTIGATIONS

Initial blood work showed hemoglobin 10.3 g/dL, leukocyte count 28,000/mm³ (later peaking at 34,000/mm³), and platelet count 75,000/mm³. Inflammatory markers were elevated: ESR 30 mm/hr and CRP >100 mg/L. Liver and renal function tests were within normal limits. Blood sugar was 104 mg/dL. Tests for malaria, dengue, enteric fever, rickettsial diseases, HIV, hepatitis B, and C were negative.

Aspiration of the thigh collection revealed purulent fluid, which cultured methicillin-sensitive *Staphylococcus aureus* (sensitive to clindamycin, vancomycin, and ceftiofuran). Ultrasound of the right thigh identified a 16 × 1.8 cm collection in the vastus lateralis with diffuse edema, confirmed by MRI as pyomyositis. Doppler ultrasound showed deep vein thrombosis involving the distal saphenofemoral and popliteal veins, consistent with septic thrombophlebitis.

Chest radiograph revealed bilateral peripheral opacities and consolidation. CT pulmonary angiography demonstrated scattered peripheral and subpleural consolidations with areas of cavitation and mild bilateral pleural effusion, indicative of septic pulmonary embolism (Fig.2,3).



Fig. 2. Chest radiograph showing bilateral peripheral radiodense shadows suggestive of consolidation.

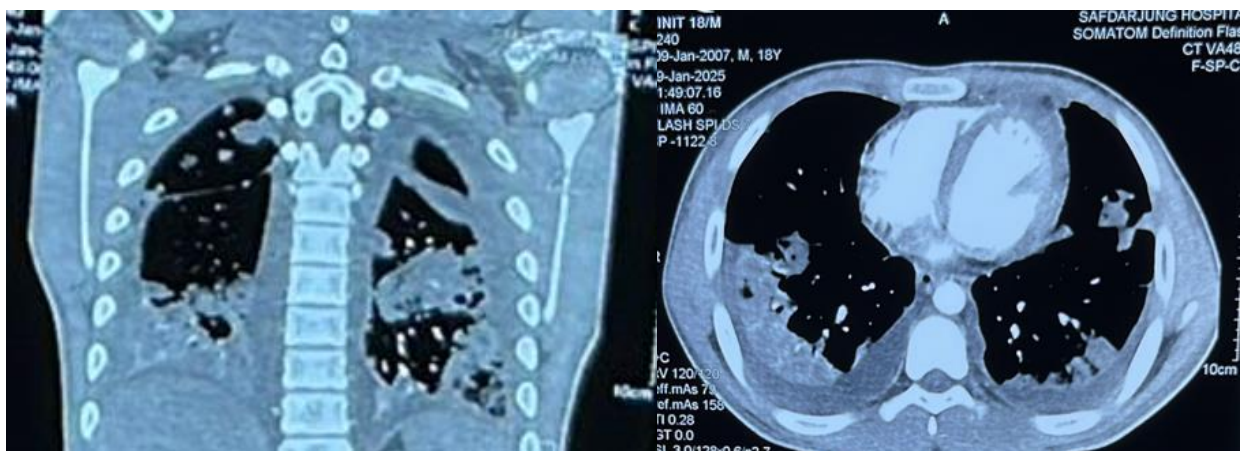


Fig. 3. CT of the thorax revealing multiple scattered consolidations with areas of breakdown in peripheral and subpleural locations, along with bilateral mild pleural effusion, suggestive of septic pulmonary embolism.

TREATMENT AND OUTCOME

Empirical broad-spectrum antibiotics were initiated and later tailored to vancomycin and clindamycin based on sensitivity. Anticoagulation was started with low molecular weight heparin, followed by oral dabigatran. Due to the progression of pyomyositis, surgical drainage was performed, yielding approximately 200 mL of pus. The patient showed rapid clinical improvement, became afebrile within five days, and maintained normal oxygen saturation on room air. Mobility progressively returned, and he was discharged in stable condition after 10 days of hospitalization. He continues to do well on follow-up.

DISCUSSION

What made this case particularly remarkable was the co-existence of seemingly unrelated septic processes, namely, pneumonia and pyomyositis, alongside deep vein thrombosis (DVT). Initially, we considered these findings to be separate complications. However, their concurrent presentation prompted us to reassess the etiology and look for a unifying diagnosis. The discovery of DVT adjacent to the inflamed thigh muscle was especially perplexing.

Septic pulmonary embolism (SPE) is an uncommon form of pulmonary embolism caused by the embolization of infected thrombi to the pulmonary arterial circulation, leading to clinical and radiological findings such as peripheral consolidations, empyema, pleural effusion, and lung abscesses. In 1978, a study reported 60 cases of septic pulmonary embolism, 78% of which occurred in intravenous drug users. Since then, with the growing use of intravascular devices and catheters, an increasing number of catheter-related SPE cases have been described in the literature (6,7)

A systematic review by Rui Ye et al. analyzed 168 cases of septic pulmonary embolism, identifying a range of primary infection sources. The most common were intravenous drug use (26.19%), followed by indwelling intravascular catheters (12.5%), infective endocarditis (11.9%), and liver abscesses (8.93%). Other significant sources included skin and soft

tissue infections (5.95%), septic thrombophlebitis (5.95%), and dental and periodontal infections (5.36%). Notably, Lemierre's syndrome accounted for 5.36% of cases, while pyomyositis was identified in only four instances, underscoring its rarity but reinforcing its potential role as an underlying cause (8).

Septic thrombophlebitis can theoretically involve any vein, whether superficial or deep. Its diagnosis is established based on clinical presentation, culture results, and radiographic evidence of venous thrombosis. A retrospective case series by Jorge A. Brenes et al. highlights that the coexistence of an extrapulmonary infection with contiguous septic thrombophlebitis and subsequent septic pulmonary embolism occurs in both adults and children (9). In this process, a localized infection allows organisms, typically bacteria, to invade the venous system, where associated edema may compress the vein and cause stasis. Once in the bloodstream, endothelial injury from toxins and inflammatory mediators, along with direct thrombotic effects of the pathogen, promotes thrombus formation. The resulting fibrin and platelet matrix provides an ideal environment for bacterial growth and propagation, serving as a source for metastatic infection as fragments embolize to the pulmonary circulation (10).

Pyomyositis is an acute infection of striated muscle that can present as a localized abscess or an aggressive, necrotic process. It can be classified as primary or secondary to a nearby or remote site of infection. Primary pyomyositis is rare, typically affecting children and adolescents, with the large muscles of the lower limbs being the most common site (11). It is especially prevalent in tropical regions such as Africa and the South Pacific, earning it the name tropical pyomyositis. The condition is more frequently seen in immunocompromised patients, though it can also arise due to local muscle factors such as trauma or vigorous exercise. It is believed that pyomyositis develops when a hematoma within a muscle following trauma becomes secondarily infected during an episode of bacteremia (12). The most common pathogen is *Staphylococcus aureus* (accounting for 70–90% of cases), while other organisms, such as *Streptococcus pyogenes*, *Neisseria gonorrhoeae*, *Enterococcus faecalis*, *Escherichia coli*, *Salmonella enteritidis*, and *Mycobacterium tuberculosis*, have also been implicated (13).

Management of this condition requires a multidisciplinary approach. In our patient, clinical improvement was achieved through culture-guided antibiotic therapy, anticoagulation, and surgical drainage. Although the role of anticoagulation in septic thrombophlebitis remains controversial, Valerio et al. have suggested a potential benefit in reducing further thromboembolic complications (14). Surgical drainage is critical for eliminating the suppurative focus and is indicated when swelling progresses with an imminent risk of compartment syndrome. The prognosis improves significantly with early diagnosis, allowing timely intervention and facilitating patient recovery.

This case is particularly notable not only for the presence of a rare clinical triad but also for its occurrence in a young, immunocompetent individual. It emphasizes the importance of maintaining a high index of suspicion and integrating subtle clinical cues when managing complex sepsis presentations. This case is the fourth such instance of this triad reported worldwide. The first was described in France in a soccer player, and two subsequent cases have been reported from India, one in an elderly diabetic male and another in a 17-year-old boy (2,15,16).

Conflict of interest

The authors declare that they have no conflict of interest.

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THE TIR DOMAIN RECOGNIZES INVASIVE PATHOGENS AND INDUCES AN IMMUNE RESPONSE

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ABSTRACT

The Toll-interleukin-1 receptor (TIR) is a conserved domain of the innate immune response and a signal transducer. TIR is a member of the pattern recognition receptors (PRRs) and is involved in the initiation of microbial-induced signals. Together with the TIR domain, Toll-like receptors (TLRs) recognize pathogen-associated molecular patterns (PAMPs). TIR participates in the interaction between TLRs and adaptors such as MyD88 and TIR domain-containing adaptor protein inducing interferon beta (TRIF), complex reactions that culminate in the activation of NF- κ B. To inhibit the immune response, bacteria and viruses can produce proteins that mimic the TIR domain to compete with endogenous adaptors. TLRs mediate the production of interferons (IFNs) which are antiviral immune proteins. TLR regulation is crucial in the inflammatory process.

KEYWORDS: *Toll-interleukin-1 receptor, domain, signal transducer, innate immune response, infection*

INTRODUCTION

Toll-interleukin-1 receptor (TIR) is a 150-amino acid immune system transducer (1). The TIR domain is a component of pattern recognition immune proteins (PRIPs) which is a conserved domain critical in the innate immune response. This domain is part of pattern recognition receptors (PRRs) that play a key role in signaling during infections.

The TIR domain is a structural region found in Toll-like receptors (TLRs). TLRs recognize components of pathogens such as bacteria, viruses, fungi, and parasites and are also involved in the synthesis of pro-inflammatory cytokines. With the TIR domain, TLRs recognize pathogen-associated molecular patterns (PAMPs), including lipopolysaccharides (LPS) from Gram-negative bacteria and viral RNAs (2). The TIR domain mediates the interaction between TLRs and adaptors such as MyD88 and TIR domain-containing adaptor protein inducing interferon beta (TRIF), activating NF- κ B and MAPK. The TIR domain is a conserved cytoplasmic component of TLRs and IL-1R receptors. TIR domains generate nicotinamide adenine nucleotide (NAD⁺)-derived small signaling molecules that could induce the regulated death of infected cells.

DISCUSSION

The TIR domain plays a key role in infections. It is a highly conserved portion present in both TLRs and IL-1 receptors (IL-1Rs). The TIR domain is crucial for activating the innate immune response, where macrophages are activated by TLRs and IL-1R ligands.

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TIR shares structural regions such as BOX1, BOX2, and BOX3, which are important for protein-protein interactions. Adaptor proteins such as MyD88 interact with the TIR domain and activate pro-inflammatory genes (3). In addition to reacting with cellular adaptors such as MyD88, the TIR domain interacts with TIRAP, TRIF, and TRAM. These are essential for activating the NF- κ B and MAPK cascades, which lead to the synthesis of pro-inflammatory cytokines (Fig.1). During infection, TLRs recognize conserved structures in pathogens such as PAMPs, including Gram-negative bacterial products that bind to TLR4 and viral RNA, which in turn binds to TLR-3, TLR-7, and TLR-8; while bacterial DNA binds to TLR-9 (4). Once the TIR domain binds to the pathogen, signal transduction occurs with the recruitment of adaptor proteins, including MyD88, and the production of IL-1 β , TNF, and interferons (IFNs).

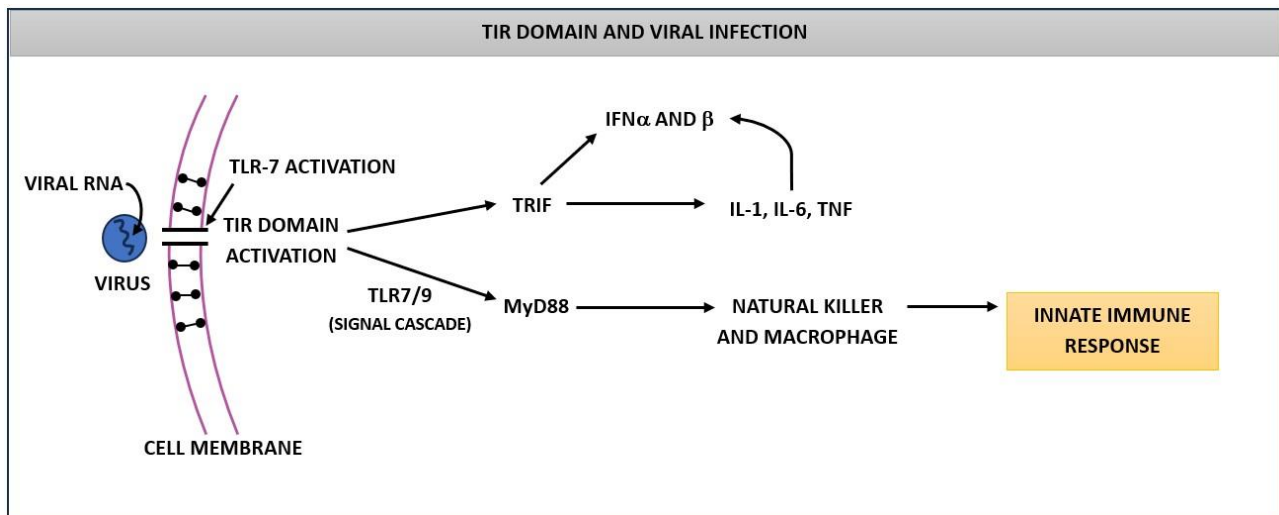


Fig. 1. Viral RNA activates Toll-like receptor (TLR)-7 which activates the Toll-interleukin-1 receptor (TIR) domain and results in a signaling cascade involving MyD88 that modulates immune cells mediating the immune response. Additionally, TIR domain-containing adaptor protein inducing interferon beta (TRIF) leads to the induction of cytokines.

However, pathogens have developed strategies to evade the TIR. Some bacteria, such as *Salmonella*, produce proteins that mimic the TIR domain, competing with endogenous adaptors and inhibiting the immune response. For example, *Brucella melitensis* produces TIR domain-containing protein B (TepB), which mimics the TIR and binds to the Toll-interleukin 1 receptor domain-containing adaptor protein (TIRAP/MAL), preventing the activation of TLR-2 and TLR-4.

Mutations in the TIR domain can lead to immunodeficiency with susceptibility to bacterial infections. Immune deficiency can also lead to chronic inflammation or autoimmunity. The intracytoplasmic TIR protein is important for immune signal transduction and plays a key role in the innate immune response, particularly during viral infections. The TLR resists viral attack by activating the interferons IFN- α and IFN- β (5).

Some viruses have developed evasion mechanisms to interfere with TIR signaling. Viruses can inhibit TLR dimerization and degrade the MyD88 and TRIF adaptors. Furthermore, some viruses can block signal transduction in adaptors. In the antiviral response, influenza viruses activate TLRs 7/8 and MyD88, which are required for the production of IFNs that trigger an immune response (6).

In coronavirus disease 2019 (COVID-19) induced by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), TLR-7 is activated, particularly in dendritic cells, causing an IFN response even though the virus can inhibit MyD88 and TRIF. Herpes viruses activate TLR-9 and subsequently, type I IFN. TLR inhibitors limit inflammation, while stimulators act as immunomodulators and increase antiviral activity.

CONCLUSIONS

TIR is an immune signal transducer and is part of the PRRs that recognize microorganisms. TIR participates in the action of TLRs that bind microorganisms and activate MyD88, TRIF, and NF- κ B, resulting in the production of immunoregulatory cytokines. Some bacteria and viruses produce proteins that mimic the TIR domain and inhibit the immune response. TLR regulation by TIR is essential for modulating acute and chronic inflammation and inflammatory diseases.

Conflict of interest

The authors declare that they have no conflict of interest.

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AUTISM SPECTRUM DISORDERS AND INFECTIONS

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ABSTRACT

Autism spectrum disorder (ASD) is a multifactorial childhood disorder in which genetic, epigenetic, environmental, and immunological components play a crucial role. The possible connection between infections and autism has recently been explored. Studies have focused on cellular and molecular mechanisms involving neurobiology, the immune response, and inflammation. Maternal viral or bacterial infections during pregnancy can activate a systemic immune response with the production of pro-inflammatory cytokines, which cross the newborn's immature blood-brain barrier and can cause brain damage. Alterations can occur in neuronal migration, synaptogenesis, and myelination, with changes in brain architecture associated with autism spectrum disorders. Neuroinflammation can negatively affect synaptic plasticity and neuronal function. Microorganisms can activate microglia, which produce pro-inflammatory cytokines and chemokines, counteracting the disease.

KEYWORDS: *Autism spectrum disorder, infection, neurobiology, inflammation, neurodevelopment*

INTRODUCTION

Autism spectrum disorder (ASD) is a varied group of neurodevelopmental conditions characterized by impaired social interaction and communication and restricted and repetitive behavior (1). Today, the Center for Disease Control and Prevention estimates that 1 out of every 110 children in America are diagnosed with ASD, and there has been an impressive rise in ASD in the last century (2). Children with ASD show complex developmental abnormalities defined on the basis of the severity of symptoms, particularly in language, socialization, learning, and stereotypical behaviors. About 30% of ASD children show sudden clinical regression at around 3 years of age. After much research by multiple large studies into the pathogenesis of this disease, it is now clear that vaccines do not cause autism. In addition, there is no single pathogen known to cause ASD and it is likely that the disease is due to a combination of different factors (3). In recent years, there has been a growing interest in the relationship between ASD and infections in general (4).

DISCUSSION

Infections can have a major impact on neurodevelopment, especially in the embryonic period and early childhood. However, studies are still unraveling the complexities of this association. It is likely that there are possible connections between infections and ASD. For example, maternal infections in the prenatal period during pregnancy have been associated with an increased risk of ASD in the child.

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There are various pathogens that may contribute to the risk of ASD. For instance, congenital rubella syndrome, caused by rubella infection during pregnancy, can lead to developmental problems and ASD behaviors. Another infectious agent, cytomegalovirus (CMV), has been associated with neurological disorders, including ASD (5). Cases of influenza and fever during pregnancy, especially in the early months, can activate the maternal immune system against the fetus and increase the risk of ASD.

The mechanisms of maternal immune and inflammatory responses that alter physiological brain function are not yet clear. However, *in vitro* and *ex vivo* experiments on the brains of deceased autistic children (samples provided by the NIH), have demonstrated that pro- and anti-inflammatory cytokines are involved in ASD (6). Pro-inflammatory cytokines are produced by microglia activated by a putative antigen that could also be a microbial agent, which could pass the blood-brain barrier (BBB) that is not yet fully formed in the child. Therefore, inflammatory cytokines could damage the brain tissue still in development.

Herpes viruses and enteroviruses have been implicated in the development of ASD following postnatal infections, especially in the first period of life when the BBB is not yet formed. Alterations of the gut microbiota due to infections could also influence the gut-brain axis and modify children's behavior (7). Dysregulation of the immune system in the child, such as autoantibodies directed against brain tissue with activation of the autoimmune response, could also contribute to the development of ASD (8). Thus, infections may impact neurodevelopment, interfering with neuronal proliferation and migration, sympathetic development, and microglia activation, all of which are phenomena that are found in ASD.

However, it is possible that infections by microorganisms alone are not sufficient to cause ASD but may interact with genetic susceptibility. For example, a child with mutations in sympathetic genes may be more vulnerable to the effects of maternal infection or immune activation. However, there is no direct evidence on the specificity of infections associated with ASD. It is likely that targeted clinical trials on specific anti-inflammatory or immunomodulatory treatments may help in understanding this serious pathology (9).

CONCLUSIONS

ASD is a neurological disorder that still raises many questions regarding its diagnosis and treatment. Recent studies have highlighted how certain maternal infections during pregnancy may cause ASD. Cytokine activation by microorganisms, which cross the BBB, can cause neuronal damage and could induce ASD. There is no single microorganism that can cause ASD, and this serious brain disorder is likely due to a combination of factors. Additionally, it is now certain that vaccines do not cause autism, as this has been widely disproved by numerous international studies. Because the pathogenesis of this disorder is completely unknown, further studies are needed to clarify this enigma.

Conflict of interest

The authors declare that they have no conflict of interest.

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INFLAMMATORY PROTEIN CASPASE-1 PLAYS A CRUCIAL ROLE IN THE IMMUNE RESPONSE DURING MICROBIAL INFECTIONS

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ABSTRACT

Caspase-1 is a proteolytic enzyme that mediates the immune response in physiological and pathological states, including infections. Activation of caspase-1 is linked to the production of cytokines. Caspase-1 induces pyroptosis by cleaving the protein Gasdermin D (GSDMD), a pore-forming protein that perforates the plasma membrane. Caspase-1 limits the replication of microorganisms by activating the innate immune response. Microorganisms activate inflammasomes that participate in the exacerbation of cytokine production, causing a worsening of the infectious state. Caspase-1 is derived from the inactive molecule pro-caspase-1 and is activated by the inflammasome complex which in turn is activated by both exogenous and endogenous danger signals to the cell, activating NOD-like receptor family, pyrin domain containing 3 (NLRP3). Caspase-1 mediates the formation of IL-1 β which, by binding to its receptor IL-1R, activates the cascade that leads to the formation of NF- κ B, with generation of inflammatory cytokines and adhesion molecules such as ICAM-1.

KEYWORDS: *Caspase-1, enzyme, infection, inflammasome, virus, bacteria*

INTRODUCTION

Caspase-1 is an important protein in the immune response to infections. This enzyme belongs to the family of cysteine protease caspases that are activated during inflammation (1). Caspase-1 is involved in the activation of inflammatory cytokines mainly derived from innate immune cells. Inflammatory caspase-1 is a proteolytic enzyme that processes precursors of pro-inflammatory cytokines such as pro-IL-1 β and pro-IL-18 (2). Furthermore, caspase-1 induces pyroptosis, or cell death, by cleaving the protein Gasdermin D (GSDMD), a pore-forming protein that perforates the plasma membrane during pyroptosis (3).

During infections caused by viruses or bacteria, caspase-1 is activated by an intracellular NOD-like receptor family, pyrin domain containing 3 (NLRP3) complex which belongs to the inflammasome family (4). The task of caspase-1 is to convert pro-IL-1 β and pro-IL-18 into their mature active forms that are highly inflammatory cytokines (5). In addition to mediating inflammation, IL-18 also mediates fever, and induces the recall of immune cells to the inflamed site after an infection (6).

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Caspase-1 activates the immune response to limit the replication of microorganisms (7). This activation is important for the containment of the infecting microbes. However, the activation of caspase-1 can also harm the host, depending on the degree of enzyme activation. Normal physiological activation induces an immune response useful to the patient, while exaggerated activation causes inflammation (8). Therefore, the effect of caspase-1 must be balanced; if it is too mild it leads to an ineffective immune response, while, if it is too strong, it can cause serious inflammatory damage.

DISCUSSION

Both bacterial and viral microorganisms activate caspase-1 (1). Some microorganisms activate certain inflammasomes, such as *Salmonella typhi*, and others, such as viruses, activate other inflammasomes (9). Inflammasomes participate in the exacerbation of cytokine production, contributing to the worsening of infection (10). Both caspase-1 and IL-1 β play a crucial role in the innate inflammatory response (11).

Caspase-1 is synthesized as an inactive zymogen (pro-caspase-1) and is activated within multiprotein complexes called inflammasomes (12). Activation of the inflammasome at the molecular level occurs through exogenous danger signals such as lipopolysaccharide (LPS) and pathogen-associated molecular patterns (PAMPs), or endogenous signals such as damage-associated molecular patterns (DAMPs) and extracellular ATP that activate inflammatory receptors, including NLRP3 (13).

IL-1 β is a key cytokine in inflammation and requires two signals to be activated (14). The first is LPS, which induces the transcription of pro-IL-1 β via nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) (15). The second is ATP, which activates the inflammasome that in turn activates caspase-1, a reaction that leads to the cleavage of pro-IL-1 β into mature IL-1 β (16). Once activated, IL-1 β binds to its receptor (IL-1R) on target cells and activates the signaling cascade via MyD88, IRAK, and NF- κ B (17). This induces the expression of other pro-inflammatory cytokines including IL-6 and tumor necrosis factor (TNF), and some adhesion molecules (e.g. ICAM-1), causing fever and systemic inflammatory responses (18).

Infected macrophages and monocytes activate NLRP3, although it is still unclear how microorganisms can trigger the activation of this multiprotein complex that forms in response to intracellular danger signals (19). NLRP3 activation has been demonstrated in virus-infected macrophages and monocytes in *in vitro* mouse models. These studies suggest that the virus triggers non-canonical caspase 4/11-mediated NLRP3 activation (20). NLRP3 is a cellular sensor that recognizes both DAMP and PAMP signals (Fig.1).

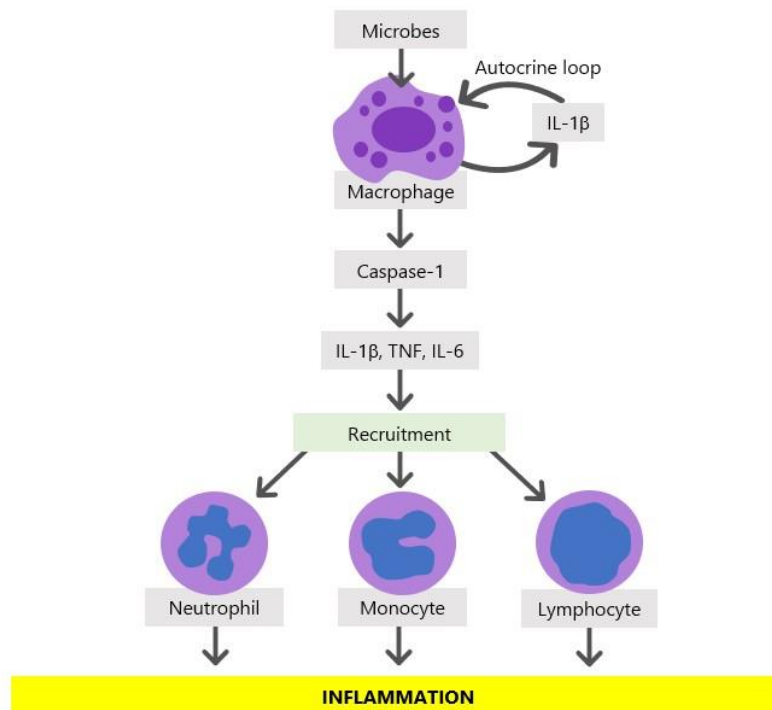


Fig. 1. Macrophages are activated by IL-1 to release inflammatory cytokines and recruit neutrophils, monocytes, and lymphocytes to the site of inflammation.

The caspase activation and recruitment domain (CARD) that connects NLRP3 to caspase-1 is not directly present on NLRP3 (21). This job is performed by the adapter protein ASC, also known as PYCARD (22). NLRP3 has an N-terminal pyrin domain (PYD) and PYCARD has two domains: an N-terminal PYD and a C-terminal CARD (23). Upon stimulation, NLRP3 oligomerizes and interacts via PYD-PYD with apoptosis-associated speck-like protein containing a CARD (ASC), creating an inflammasomal “speck” (24). These reactions allow the recruitment and activation of procaspase-1 into active caspase-1.

Transcription of pro-IL-1 β , NLRP3, and other pro-inflammatory proteins is induced by signals such as Toll-like receptors (TLRs) that activate NF- κ B (25). Other secondary activating stimuli include extracellular ATP, reactive oxygen species (ROS), urate crystals, bacterial toxins, and others that cause NLRP3 oligomerization and the formation of the inflammasome complex (26). The complex activates caspase-1, which matures IL-1 β and IL-18, induces pyroptosis (via GSDMD) (27). Thus, caspase-1 induces a rapid response to infections, with control of tissue repair and immune response (28).

Excessive activation causes chronic inflammatory diseases such as gout (caused by monosodium urate), atherosclerosis, autoimmune diseases (such as lupus and rheumatoid arthritis), metabolic diseases (type 2 diabetes), neurodegenerative diseases (Alzheimer's and Parkinson's), amongst others (29).

The NLRP3 complex is an important alarm signal that activates caspase-1, triggering the inflammatory response mediated by cytokine activation that promotes pyroptosis (30). Inhibition of caspase-1, and hence of IL-1 β , could be helpful in the therapy of chronic infectious diseases (31).

CONCLUSIONS

Caspase-1 is an inflammatory caspase that plays a critical role in innate immunity and activates pro-inflammatory cytokines such as IL-1 β and IL-18 by cleaving their precursors. Caspase-1 is a proteolytic enzyme derived from procaspase-1 (inactive) that is activated in infectious processes where an inflammatory state mediated by IL-1 β is generated. Caspase-1 limits the replication of microorganisms by activating the innate immune response, but its overproduction increases the levels of IL-1 β that activates the recruitment of immune cells with the formation of other pro-inflammatory cytokines. Inhibiting caspase-1, and therefore, IL-1 β , could have therapeutic value for some chronic infectious diseases.

Conflict of interest

The authors declare that they have no conflict of interest.

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THE PROTEASOME IS CRUCIAL FOR THE DEGRADATION OF VIRAL AND BACTERIAL PROTEINS

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KEYWORDS: *Proteasome, virus, bacteria, infection, inflammatory response*

INTRODUCTION

The 26S proteasome is an intracellular protein complex implicated in the immune response against infections that is responsible for the degradation of viral and bacterial proteins (1). This proteasome exerts its biological action on ubiquitin-tagged proteins, degrading them into small peptides (2). This effect is crucial for the recycling of damaged proteins, the regulation of cell signaling proteins, and the control of the cell cycle and apoptosis. The proteasome degrades both viral and bacterial proteins in infectious diseases.

DISCUSSION

The 26S proteasome, which recognizes the polyubiquitin chain, is composed of a 20S core and two 19S regulatory subunits (3). The latter recognize the removed ubiquitin chain and unwind target proteins, translocating them to the 20S catalytic core. In degradation, the 20S core has protease activity (via the subunit) similar to chymotrypsin, trypsin, and peptide-glutamyl peptidases. The protein is degraded into peptides of approximately 8–10 amino acids (4).

When a virus or intracellular bacterium infects a cell, the proteins are released into the cytosol and translated within the host cell. The proteins are recognized as foreign and degraded by the proteasome. The peptides resulting from degradation are transported to the endoplasmic reticulum via the transporter associated with antigen processing (TAP) and loaded onto major histocompatibility complex class I (MHC-I) (5). These peptides are exported to the cell membrane and recognized by CD8⁺ lymphocytes. This mechanism is important for the immune system and triggers a cytotoxic response (Fig.1).

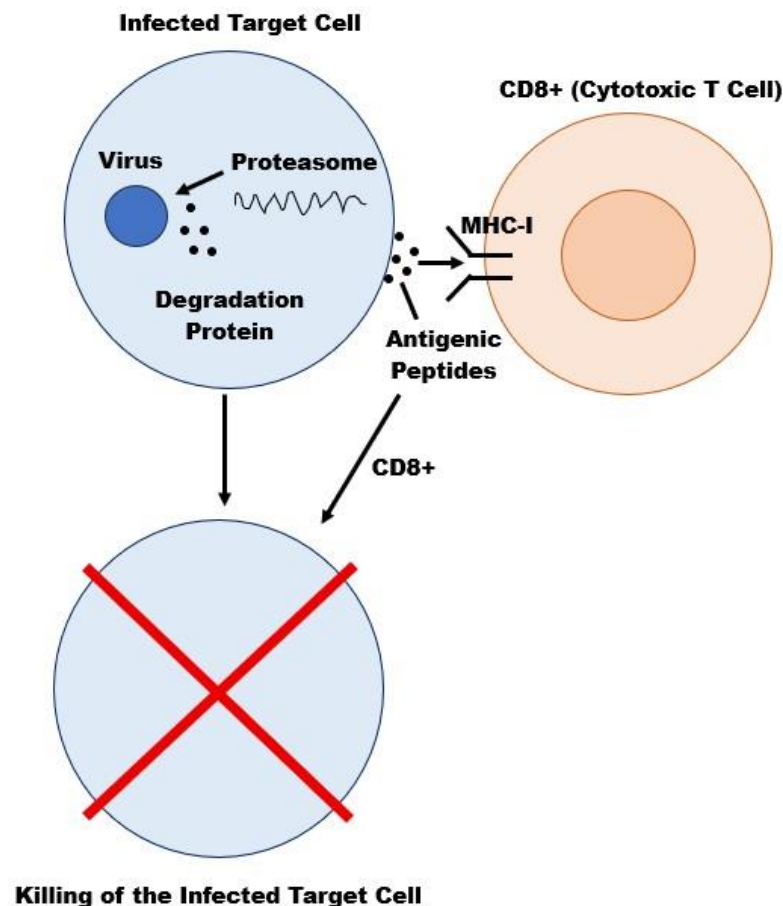


Fig. 1. When the target cell is infected by intracellular viruses, proteasomes degrade viral proteins. Antigenic peptides from the degraded proteins are exposed on the target cells and are recognized by major histocompatibility complex class I (MHC-I) on CD8+ cells. When activated, CD8+ immune cells kill the viral-infected target cells.

After degradation, the peptides are transported to the endoplasmic reticulum via TAP, where they bind to MHC-I, which exposes the peptides to the cell surface. At this point, cytotoxic cells recognize these antigenic peptides and lyse virus-infected cells. During inflammation, the immunoproteasome facilitates the production of peptides for antigen presentation.

In the inflammatory response, the proteasome regulates the degradation of the inhibitory protein inhibitor of kappa B (I κ B). The 26S proteasome plays an important role in controlling the quality of proteins, including viral and bacterial proteins which enter the cytosol and are tagged with ubiquitin, a small protein covalently linked to lysine residues of the target protein. Tagging occurs through the action of three enzymes: E1, E2, and E3 (6). E1 activates ubiquitin, E2 conjugates ubiquitin, and E3 creates a specific substrate binding that confers specificity.

Viruses and bacteria can activate an evasive strategy to avoid their lysis. For example, they can inhibit the ubiquitin-proteasome system. Another method of microbial evasion involves inhibiting TAP or even preventing peptide loading onto MHC-I.

CONCLUSIONS

In conclusion, the 26S proteasome plays an important role in the immune response against intracellular viruses and bacteria by degrading pathogenic proteins and contributing to antigen presentation. In the immune response against viruses, the proteasome is crucial for protein processing and the production of antigenic peptides that are presented to MHC-I and recognized by cytotoxic CD8+ cells.

Conflict of interest

The author declares that they have no conflict of interest.

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CAR-T GENE THERAPY WITH LENTIVIRAL AND γ -RETROVIRAL VECTORS MAY INCREASE THE RISK OF INFECTIONS

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ABSTRACT

Chimeric antigen receptor T cell (CAR-T) therapy uses viral vectors such as lentiviruses and γ -retrovirals that are used in to insert genetic material into host cells. The vectors must possess certain characteristics that allow them to be effective without harming the host cell. γ -retroviral vectors that are derived from the murine leukemia virus act on already activated cells. Lentiviral vectors are derived from the human immunodeficiency virus (HIV)-1 and carry out transduction on both proliferating T lymphocytes and on quiescent, non-activated T lymphocytes. The drugs tisagenlecleucel and axicabtagene ciloleucel are currently approved for CAR-T gene therapy. The modified T cells from patient's express an anti-CD19 CAR receptor and attack tumor B cells that express CD19, destroying them. However, this treatment induces the production of inflammatory cytokines, and neurotoxicity, especially when axicabtagene ciloleucel is used. Among the side effects with this therapy is immunosuppression that favors the onset of infections. The inhibition of immunity occurs both on the production of immunoglobulins and on phagocytosis, favoring the proliferation of microorganisms such as viruses, bacteria, and fungi. It can be concluded that despite the efficacy of CAR-T therapy, side effects are still a problem to be resolved.

KEYWORDS: *Chimeric antigen receptor T cell, CAR-T therapy, lentiviral vector, γ -retroviral vector, gene therapy*

INTRODUCTION

The study of virology and human immunodeficiency virus (HIV)-1 has been instrumental in the development of novel therapies (1). Lentiviral and γ -retroviral vector therapy have aroused much interest in the scientific community and could hold great therapeutic promise (2). These therapies consist of inserting a new gene into the DNA of the abnormal cell to compensate for the non-functioning or absent one.

Gene therapy is now being applied in the treatment of incurable genetic diseases, and these therapies raise great hopes for their treatment, even if the side effects are not yet fully understood (3). The method of replacing the diseased gene(s) with a healthy one begins with the collection of stem cells from the patient's bone marrow carrying the switched-off or absent gene, which is then put into culture. A vector virus carrying the healthy gene is inserted into these cells, generating a genetic transformation (4). The modified cells are then re-inoculated into the blood of the patient who has the gene defect. The vectors carrying the healthy gene can be lentiviral or γ -retroviral.

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In chimeric antigen receptor T cell (CAR-T) gene therapy, lentiviral and γ -retroviral vectors are important to transfer therapeutic genes into the patient's T lymphocytes (5). In gene therapy, vectors must have certain characteristics, such as acting on target cells, having stability in integration, having a low degree of oncogenic risk, having simple and inexpensive production, and allowing a wide clinical use of CAR-T (6). Lentiviral vectors are currently preferred for most CAR-T therapies due to their greater efficiency and relative safety. γ -retroviral vectors are less suitable for CAR-T therapy and carry a slightly higher risk of insertion, which can prove toxic. However, both vectors have been essential to the history and development of *ex-vivo* gene therapies, particularly in modifying T lymphocytes to combat various diseases that respond poorly to current therapies, including cancer.

DISCUSSION

In gene therapy, the first viral vectors used were retrovirals, mainly derived from the Moloney murine leukemia virus (7). These vectors integrate well into the genome and exert a long duration on the transplanted gene. In addition, γ -retroviral vectors have a preference for activated T cells, which are necessary in CAR-T therapy (8). However, γ -retroviral vectors are not able to exert transduction in non-activated resting cells, and this can sometimes be a disadvantage. Furthermore, gene insertion could potentially occur near oncogenes causing mutagenesis and tumors (9).

In recent years, there has been growing interest in the application of CAR-T cells not only in cancer, but also in chronic and resistant viral infections. This research is still in the preclinical phase; however, CAR-T cells are being studied for various resistant and chronic viral infections. For example, in acquired immunodeficiency syndrome (AIDS), the primary goal is to eliminate HIV-infected CD4⁺ cells, as well as the macrophages that serve as reservoirs of the virus. CAR-T cells recognize the viral envelope proteins gp120 that are expressed on infected cells and eliminate them in combination with anti-retroviral therapies (ART). However, infected cells may express low levels of antigen, making them difficult to recognize. In Epstein-Barr Virus (EBV), CAR-T cells can also be directed against viral proteins expressed by EBV-positive transformed cells (such as LMP1 and LMP2), while anti-cytomegalovirus (CMV) CAR-T cells are being studied in CMV infections.

Lentiviral vectors are derived from HIV-1 but are non-pathogenic (10). They exert transduction on both proliferating T cells and resting non-activated T cells (11). These vectors exert stable and long-term integration on the CAR genome and reduce the risk of reactivation and recombination. The limitation of the use of lentiviral vectors is due to their high cost, and since they derive from HIV, they require great care in their use (12).

Tisagenlecleucel and axicabtagene ciloleucel using CAR-T are approved for gene therapy today. These drugs utilize slow viral or γ -retroviral vectors based on the biological characteristics of the patient (13). Both tisagenlecleucel (brand name Kymriah) and axicabtagene ciloleucel (brand name Yescarta) are made from the patient's own (autologous) T cells that have been genetically modified to express an anti-CD19 CAR receptor, targeting B tumor cells (14). The choice of vector must be made based on the type of cell, safety, regulation, required duration of expression, and costs.

However, there are important differences between the two compounds. At the clinical level, Tisagenlecleucel exerts a greater persistence of CAR-T cells over time, while axicabtagene ciloleucel gives a faster and shorter-lasting response (15).

Tisagenlecleucel is used for B-cell acute lymphoblastic leukemia in patients over 25 years of age, while axicabtagene ciloleucel has been approved for follicular lymphoma in relapsed or refractory cases (16). However, both treatments can cause side effects such as cytokine release syndrome and can cause neurotoxicity (17). It seems that treatment with axicabtagene ciloleucel, compared to that with tisagenlecleucel, is faster and has a greater effect in triggering the production of pro-inflammatory cytokines and inducing neurotoxicity since it acts on CD28 (18).

CAR-T therapy is an advanced form of immunotherapy primarily used to treat certain types of blood cancers such as leukemia and lymphoma (19). While this therapy is very effective in some patients, it also carries risks, including a significantly increased risk of infections (20). The risk of increased infections with CAR-T therapy may depend on lymphodepletion before therapy, since before the infusion of the CAR-T cells, the patient receives chemotherapy necessary to reduce the number of lymphocytes (21). This is to create space for the CAR-T cells, generating immunosuppression (22).

CAR-T cells targeting CD19 (as in B-cell lymphoma therapies) also react against healthy B cells, reducing their number and causing hypogammaglobulinemia, which leads to infections by microorganisms (23). CAR-T therapy also induces a reduction in the number of neutrophils, elements necessary for bacterial phagocytosis, the immune system's first step against microbial infections (24).

In addition, in the case in which pro-inflammatory cytokines are induced by CAR-T treatment, the therapeutic use of corticosteroids to reduce inflammation can lead to the inhibition of the immune system and increase vulnerability to

infections (25). The most common infections that can occur during treatment with CAR-T therapy are bacterial, viral, and fungal (26). Bacterial infections occur mainly in the first 30 days of treatment, with pneumonia and urinary tract and blood infections (26). Viral infections can be due to reactivation of latent viruses such as *Herpes simplex* and *zoster*, CMV, respiratory syncytial virus (RSV), influenza, and adenovirus. Fungal infections such as aspergillosis and candidiasis are less common and are due to neutropenia.

CONCLUSIONS

CAR-T therapy uses viral vectors such as lentiviruses and γ -retroviruses. Tisagenlecleucel and axicabtagene ciloleucel are two drugs that have currently been approved for CAR-T gene therapy. Although CAR-T therapy is a promising treatment, attention needs to be focused on reducing potential negative side effects, such as the production of inflammatory cytokines, neurotoxicity, and immunosuppression that favors infections by microorganisms.

Conflict of interest

The author declares that they have no conflict of interest.

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HODGKIN LYMPHOMA, EPSTEIN-BARR VIRUS, AND CAR-T THERAPY

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ABSTRACT

Hodgkin lymphoma (HL) is a type of cancer that originates from B lymphocytes, and is one of the most common cancers in young people. Epstein-Barr virus (EBV) infection plays a significant role and increases the risk factor in approximately one-third of affected patients. Treatment of HL generally relies on a combination of chemotherapy and, in some cases, radiation therapy. Chemotherapy and immunotherapy have revolutionized treatment, leading to cure rates in nearly 80% of cases. In recent years, immunotherapy has greatly improved the disease outcome. Immunotherapy with chimeric antigen receptor t-cell therapy (CAR-T) and novel bispecific antibodies (bsAbs) has reduced the risk of relapse and increased survival in lymphoma patients.

KEYWORDS: *Hodgkin lymphoma, Epstein-Barr virus, CAR-T therapy, immunotherapy, tumor, lymphocyte*

INTRODUCTION

Hodgkin lymphoma (HL) is a malignant tumor of the lymphatic system which originates from B lymphocytes (1), a type of white blood cell essential to the adaptive immune system and originate and mature in the bone marrow where they develop to produce antibodies (Fig.1). The disease is characterized by the uncontrolled growth of lymphocytes that are essential for defending the body against foreign agents. This lymphoma is distinguished from other types of non-Hodgkin lymphoma (NHL) by the characteristic presence of Reed Sternberg cells which are visible under the microscope (2).

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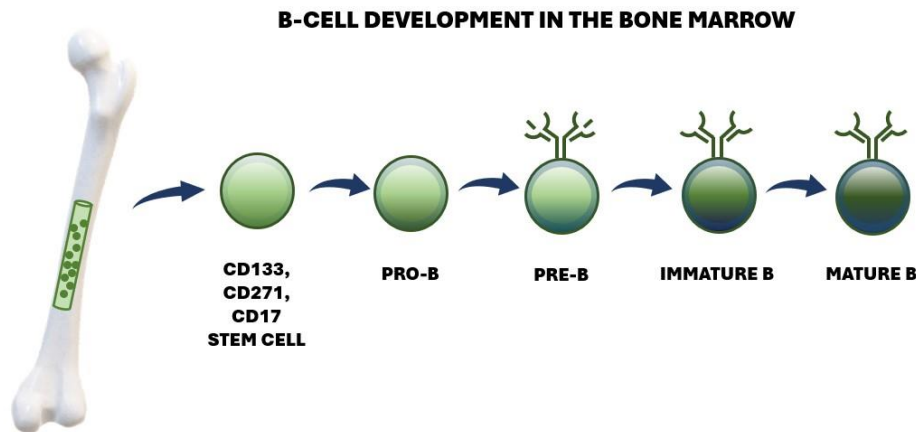


Fig. 1. The generation of mature B cells from bone marrow stem cells.

In Italy, there are approximately 1,200 new diagnoses of HL per year, with a higher incidence among young people, especially in the 15 to 30 years old age group (3). The most common symptoms include swollen lymph nodes that are often located in the neck, armpits, and/or groin, persistent fever, especially in the evening and without apparent cause, heavy night sweats, unexplained weight loss, fatigue, and widespread or localized itching (4).

Treatment of HL depends on the stage of the disease. In general, treatment is based on a combination of chemotherapy and in some cases, radiotherapy (5). A crucial contribution to improving treatment came from the Italian oncologist Gianni Bonadonna who devised the basic chemotherapy regimen still used today in the treatment of HL (6). In recent years, research has made further progress (7). For the forms most resistant to traditional treatments, the introduction of immunotherapy has represented a turning point, significantly improving the prospects for treatment. Currently, thanks to the integration of different therapeutic strategies, recovery is possible in almost 80% of cases of HL (8).

DISCUSSION

There is no specific cause for the development of HL, but some factors can increase the risk (9). Among these, the Epstein-Barr virus (EBV) plays a significant role in about a third of cases (10). This virus that causes mononucleosis can infect lymphocytes and alter their behaviour, promoting tumour development (11). However, the presence of EBV is neither necessary nor sufficient to cause HL (12). Many patients with HL show no traces of the virus, while most people infected with EBV do not develop lymphoma (13).

Lymphomas represent a significant part of the diagnoses of blood diseases and are divided into two large categories: HL and NHL. Fortunately, thanks to progress in research, the outlook for patients has improved significantly (14). The arrival of increasingly effective and targeted therapies, such as immunotherapy, chimeric antigen receptor t-cell therapy (CAR-T), and new bispecific antibodies (bsAbs), have reduced the risk of relapses and increased survival and the chances of recovery (15).

HL mainly affects young people, with a peak incidence around the age of twenty, but it can also appear at an advanced age. Over 80% of patients survive five years after diagnosis, thanks to available therapies (7). On the other hand, NHLs are more common in people over age 60 and include heterogeneous forms (16). Although they progress rapidly, the aggressive forms offer greater chances of recovery compared to the so-called indolent lymphomas, which evolve more slowly and are more difficult to eradicate (17).

EBV is associated with several cancers, especially with mixed cellularity and lymphocyte depletion subtypes, including HL (18). This association involves complex biochemical and cellular mechanisms. EBV is known to cause infectious mononucleosis and is a member of the gamma-herpesvirus family that infects B cells and epithelial cells (19). EBV causes a latent infection in B lymphocytes (20). In type II latency, HL expresses Epstein-Barr nuclear antigen 1 (EBNA1), latent membrane protein (LMP)1, and 2A-B (21). These proteins serve for proliferation and allow survival and protection against immune cells (22).

LMP1 is an oncogenic protein and develops similar functions to CD40, a constitutive factor of B cells involved in the activation of the NF- κ B pathway (23). LMP1 activates TNF receptor-associated factors (TRAFs) implicated in the nuclear translocation of NF- κ B (24). These reactions upregulate genes that promote cell survival and inflammation (25). In

addition, LMP1 is implicated in the synthesis of IL-6 and IL-13, cytokines that activate the JAK/STAT signaling pathway, promoting proliferation and immune evasion (26). Through the PI3K/AKT pathway, LMP1 is involved in apoptosis and metabolic changes that promote tumor cell proliferation (27). LMP2A is implicated in cell survival by mimicking the B-cell receptor's (BCR) function (28). This allows B-cell survival even without BCRs, which are often downregulated or nonfunctional in Hodgkin-Reed-Sternberg cells (29). LMP2A activates SYK kinases and the PI3K pathway, promoting cell viability and immune evasion (30). In HL, malignant cells produce the cytokines IL-10 and TGF- β that suppress the immune system (31).

Therapy

In general, today's therapy against lymphomas can include immunotherapy, CAR-T therapy, or bsAbs (32). Immunotherapy works by boosting the immune system, removing the brakes that the tumor uses to escape the control of natural defenses. This is a strategy that makes the immune system more effective in recognizing and attacking malignant cells (33).

CAR-T therapy is a personalized and innovative technique (34). The patient's T lymphocytes are removed, genetically modified to recognize a specific target present on the tumor cells, and reinfused into the body (35). These enhanced cells multiply and attack the tumor with precision (Fig.2).

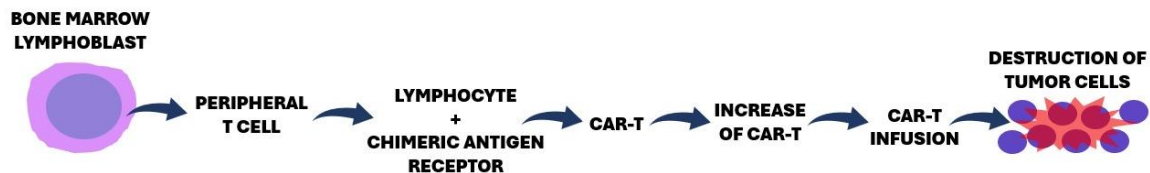


Fig. 2. Mechanisms involved in the generation of chimeric antigen receptor t-cell therapy (CAR-T) to destroy tumor cells.

BsAbs are engineered antibodies designed to bind to two different antigens (epitopes) at the same time (36). They differ from conventional monoclonal antibodies that target only one antigen. This ability to attack a dual target gives them unique therapeutic advantages, especially in cancer immunotherapy (37). The bsAbs are a further evolution of immunotherapy. They bind simultaneously to the tumor and healthy T lymphocytes, putting them in direct contact (38). This stimulates the targeted response that allows the lymphocytes to selectively eliminate cancer cells (Fig.3).

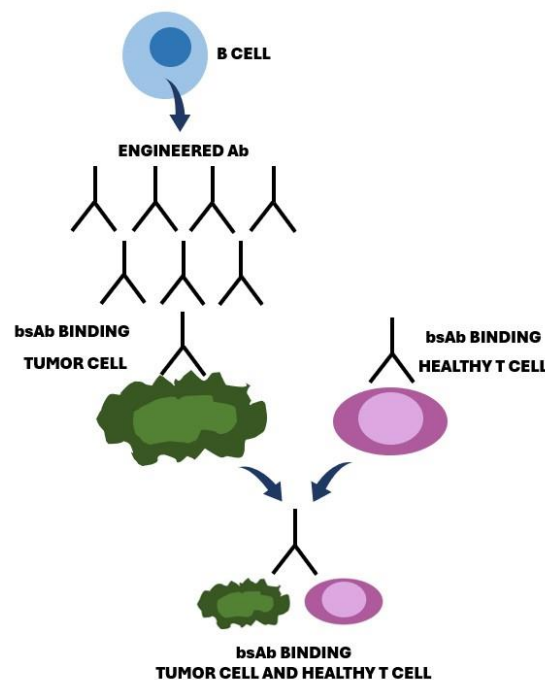


Fig. 3. Bispecific antibodies (bsAbs) are engineered antibodies produced by B cells that bind tumor and healthy cells.

In recent years, research has transformed the therapeutic landscape (39). New strategies such as immunotherapy, CAR-T therapy, and bsAbs are significantly improving patients' outlook (38). For diffuse large B-cell lymphomas, which represent approximately 30% of NHLs, new options are available that can reduce the risk of disease progression and increase the chances of recovery (40). Progress continues even for patients with relapsed or refractory forms. BsAbs are capable of activating the immune system to distinguish tumor cells and are one of the most promising innovations. These treatments are often administered in a less invasive way than traditional therapies, and lasting responses are being obtained even in very complex clinical situations. Today, the challenge is not only to develop more effective treatments but to make them available to all patients, while ensuring an adequate quality of life (41). The latest generation of therapies are helping to improve both of these dimensions, offering new hope to those facing a diagnosis of blood cancer, including HL.

CONCLUSIONS

HL is a cancer of the lymphatic system that originates from B lymphocytes and primarily affects young people. Its origin is unknown, but EBV infection plays an important role. Immunotherapy with CAR-T cells and bsAbs is generating great expectations for treating HL.

Conflict of interest

The authors declare that they have no conflict of interest.

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THE ROLE OF CASEINOLYTIC PEPTIDASE B IN CYTOKINE-MEDIATED INFLAMMATION

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ABSTRACT

Caseinolytic peptidase B (ClpB) is a chaperone ATPase enzyme that mediates many biological processes, including the generation of inflammatory cytokines. The bacterial protein ClpB plays a crucial role in the cellular stress response, particularly heat stress. ClpB belongs to the Enterobacteriaceae family, which also includes *Escherichia coli*. ClpB is involved in bacterial survival and resistance by restructuring denatured proteins and it activates macrophages to produce the inflammatory cytokines TNF and IL-6. The immune response to microorganisms generates interferon-gamma (IFN- γ), which helps eliminate Δ ClpB mutants. ClpB is highly immunogenic and could be a valuable diagnostic tool. During host stress, ClpB promotes protein recovery and adaptation and interacts with the host. It can be secreted or exposed to the surface, triggering the release of cytokines. It dysregulates proteins involved in specific functions, such as pigmentation, inflammation, energy homeostasis, and sexual function. The hypothalamic-pituitary-adrenal (HPA) axis pathway regulates the body's response to stress and leads to the production of hormones such as cortisol. Microbiota modulate the HPA axis through immune, metabolic, and nervous system mechanisms. ClpB contributes to systemic inflammation by inducing autoantibodies and can alter cortisol release.

KEYWORDS: Caseinolytic peptidase B, ClpB, ATPase enzyme, cytokine, bacteria, immune response

INTRODUCTION

Caseinolytic Peptidase B (ClpB) is a chaperone ATPase involved in the stimulation of host cytokines (1). It is a ring-shaped hexamer with two NBD domains (AAA+) in each monomer and a regulatory intermediate domain (2). ClpB is a bacterial protein that is implicated in host-pathogen interactions and modulates cytokine activity in the immune system. ClpB is a heat shock chaperonin produced by some bacteria of the Enterobacteriaceae family, including *Escherichia coli* (3) that is linked to the intestinal microbiota and affects metabolic, neuropsychiatric, autoimmune, and inflammatory disorders (4).

ClpB is a Hsp100-type chaperone protein produced by commensal bacteria such as *E. coli*. The BClpB protein is involved in the survival of bacteria by helping them survive environmental stress by restructuring denatured proteins (5). For some bacteria, such as *Mycobacterium tuberculosis*, ClpB is essential for stress tolerance and latent survival (6). ClpB is purified from *M. tuberculosis* and also acts as a chaperone, binding to macrophages and inducing the release of pro-inflammatory cytokines such as TNF and IL-6 (6).

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DISCUSSION

ClpB promotes bacterial survival and directly modulates host immunity (7). In infections, ClpB promotes the survival of various pathogens under conditions of host-induced stress-heat, oxidative stress, and nutrient starvation, and its absence often leads to attenuated virulence in animal models (8).

For example, in *Francisella tularensis*, a small ($\approx 0.2 \times 0.7 \mu\text{m}$) aerobic, facultatively intracellular Gram-negative bacillus, ΔClpB mutants are eliminated more rapidly and stimulate a protective immune response to interferon-gamma (IFN- γ) (9). *F. tularensis* is a bacterium belonging to the Francisellaceae family that is one of the most infectious microorganisms, and causes tularemia, an anthroponosis (10). It is a nonmotile, nonspore-forming, encapsulated coccobacillus, that is oxidase and catalase positive. *F. tularensis* is an intracellular parasite that infects various animals and can also be transmitted to humans via vectors such as cats, ticks, insects, and other parasites (11).

ClpB possesses immunogenicity and diagnostic potential that is similar to that found in leptospirosis, where the ClpB gene from *Leptospira interrogans* (ClpBLi) is expressed during infection and is highly immunogenic (12). In this case, animals produce specific antibodies, suggesting its potential as a diagnostic antigen. *Leptospira*, which is "leptos" in Greek (meaning "thin"), is classified into *L. interrogans* and *L. biflexa*, which comprise 60 serotypes grouped into 28 serogroups, including the saprophytic, non-pathogenic *Leptospira* that live freely in water (13). The species *L. interrogans* comprises more than 250 different serotypes or serovars that are divided into 24 serogroups, all of which are considered pathogenic (14). The disease caused by this bacterium is known as leptospirosis, which is a major zoonosis spread worldwide.

Humans express a mitochondrial homologue of ClpB that is also part of the AAA⁺ family, but it acts intracellularly and is not secreted as a cytokine stimulator (15). ClpB plays a dual role in infection; at the intrabacterial level, it promotes protein recovery and adaptation during host stress, and it also interacts with the host and can be secreted or surface-exposed, triggering cytokine release and immune responses (16).

Studies demonstrate that ClpB is a virulence factor and a potential target for new therapies or vaccines (17). This protein from intestinal bacteria, such as Enterobacteriaceae, structurally mimics the host neuropeptide α -melanocyte-stimulating hormone (α -MSH), which plays a role in homeostasis and inflammatory responses by regulating cytokine production via melanocortin (MC) receptors (18). The molecular mechanisms of α -MSH mimicry is involved in IL-6 and IL-10 dysregulation through the MC signaling pathway (19). MC receptors are a family of G-protein-coupled receptors (GPCRs) that are distinguished into five subtypes: MC1R-MC5R, each with specific functions that affect pigmentation, inflammation, energy homeostasis, and sexual function, amongst others (20,21). Mutations in the MC4R gene are the most common cause of obesity and are found in approximately 1-6% of severe obesity cases (22). MC4R is found primarily in the paraventricular nucleus of the hypothalamus, the brainstem, and sympathetic motor neurons. It contributes to the regulation of appetite, energy metabolism, cardiovascular function, reproduction, pain, mood, and sexual function (23).

Gut-brain immune signaling involves the cytokines IL-1 β and IL-6, through the hypothalamic-pituitary-adrenal (HPA) axis pathway and vagus nerve (24). HPA is a neuroendocrine system that regulates the body's response to stress that is made up of three main components: the hypothalamus, the pituitary gland, and the adrenal glands (25). The HPA produces hormones which help the body respond to environmental stressors, such as cortisol (26). The microbiota modulates the HPA axis through immune, metabolic, and nervous system mechanisms (e.g., SCFAs, LPS, peptidoglycans, vagus nerve) (27). By inducing autoantibodies, ClpB contributes to systemic inflammation and can alter cortisol release (28). ClpB has been used as a biomarker for microbiota-related disorders with immune components (4).

ClpB is an important chaperone present in bacteria and in mitochondrial homology in eukaryote cells (such as Skd3/CLPB in humans) (29). ClpB causes resistance in microorganisms surviving in hostile conditions generated by the inflammatory response, including high temperature, variable pH, and oxidative and nitrosative stress (30). In *M. tuberculosis*, cytosolic ClpB is released and interacts with macrophages, modulating the inflammatory response and contributing to the stability of tuberculosis granulomas (31,32). The release of ClpB from the bacterial cytoplasm can also activate macrophage cells in infections caused by *Mycoplasma pneumoniae*, *L. interrogans*, and *F. tularensis* (33).

CONCLUSIONS

ClpB is a chaperone in the Enterobacteriaceae family and mediates numerous biological processes. It plays a crucial role in bacterial responses to cellular stress and in the immune response, contributing to the elimination of ΔClpB mutants. In innate immunity, ClpB activates macrophages to produce inflammatory cytokines. ClpB may be a valuable marker in microbiota-related immune diseases.

Conflict of interest

The authors declare that they have no conflict of interest.

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