Brief Report

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Acute Molecular Response Post ACL Innjury: Connection to AMI and Motor Control

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Abstract: Anterior cruciate ligament (ACL) injuries are common, particularly among athletes, leading to significant functional impairment, prolonged rehabilitation, and an elevated risk of secondary complications. The ACL plays a critical role in knee stabilization, and its rupture initiates a cascade of molecular and neuromuscular changes that disrupt joint stability and motor control. One of the primary consequences of an ACL injury is the onset of Arthrogenic Muscle Inhibition (AMI), a neurological phenomenon characterized by reduced voluntary muscle activation, particularly in the quadriceps, resulting from altered afferent feedback, nociceptive inputs, and local inflammatory responses. The acute phase is dominated by pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α, which propagate local inflammation, sensitize nociceptors, and disrupt both spinal and supraspinal motor pathways. This leads to central and peripheral sensitization, exacerbating AMI and impairing rehabilitation. As the injury progresses, an anti-inflammatory shift marked by IL-10 and IL-4 promotes tissue repair and resolution of inflammation. However, dysregulation in this transition can result in chronic inflammation and fibrosis, further complicating recovery. Understanding the temporal dynamics of these molecular mediators is essential for developing targeted interventions. This review explores the complex interplay between cytokine profiles and neuromuscular adaptations post-ACL injury, highlighting potential therapeutic targets and personalized rehabilitation strategies that could optimize functional recovery and mitigate long-term complications.

Keywords: anterior cruciate ligament; molecular biology; knee joint

Introduction

Anterior cruciate ligament (ACL) injuries are among the most common and debilitating musculoskeletal injuries, particularly in athletes and active individuals, resulting in substantial loss of function, prolonged rehabilitation, and a high risk of secondary complications. The ACL is a key stabilizer of the knee joint, preventing excessive anterior translation and rotational movements of the tibia relative to the femur. Its disruption not only compromises joint integrity but also initiates a cascade of complex pathophysiological processes that extend far beyond the local damage to the ligament itself. An ACL tear often triggers rapid joint swelling, intra-articular bleeding, and the release of a multitude of damage-associated molecular patterns (DAMPs) and pro-inflammatory cytokines that contribute to a highly reactive synovial environment. These molecular changes set off a chain reaction that impacts not only the joint capsule and surrounding soft tissues but also the neuromuscular system, resulting in widespread alterations in muscle activation patterns and motor control.

One of the most notable consequences of an ACL injury is the onset of Arthrogenic Muscle Inhibition (AMI), a neurological phenomenon that manifests as a significant reduction in voluntary muscle activation, particularly affecting the quadriceps muscle group. AMI is thought to arise from a combination of altered afferent feedback, nociceptive inputs, and local inflammatory changes within the joint, which collectively inhibit the excitability of alpha motor neurons in the spinal cord

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and disrupt normal corticospinal and spinal reflex pathways. This inhibition is primarily driven by reflexive changes that are initiated almost immediately post-injury, including the downregulation of sensory feedback pathways, increased activity of inhibitory neurotransmitters, and modifications in cortical excitability. AMI leads to impaired recruitment of the quadriceps muscles, reduced force production, and difficulties in executing complex motor tasks, which significantly impede rehabilitation efforts and delay recovery. As a result, even after surgical repair and physical therapy, many individuals experience persistent muscle weakness, proprioceptive deficits, and an increased risk of secondary joint injuries, such as meniscal tears or osteoarthritis, which further complicate the recovery process.

The underlying molecular mechanisms driving these neuromuscular alterations are multifaceted and involve a dynamic interplay between pro-inflammatory and anti-inflammatory cytokines, chemokines, growth factors, and neurotrophic molecules that evolve over time. Immediately following ACL rupture, the acute inflammatory response is dominated by the release of pro-inflammatory cytokines such as interleukin-1 beta (IL-1β), IL-6, and tumor necrosis factor-alpha (TNF-α), which not only propagate local inflammation but also sensitize peripheral nociceptors, enhancing pain perception and contributing to the onset of AMI. This heightened state of nociception further disrupts motor control by altering the excitability of both spinal and supraspinal circuits, leading to increased inhibition of quadriceps activation and the development of compensatory movement patterns that further stress the knee joint. As the injury progresses into the subacute and chronic phases, a shift toward an anti-inflammatory profile, characterized by cytokines such as IL-10 and IL-4, is necessary for resolving inflammation and promoting tissue repair. However, this transition is often incomplete or dysregulated in the context of ACL injuries, leading to chronic inflammation, fibrosis, and persistent muscle inhibition.

The consequences of these molecular and neuromuscular changes are profound, as they create a vicious cycle where impaired muscle activation leads to altered joint mechanics, increased joint loading, and the potential for further joint degeneration. Understanding the precise molecular drivers of these processes is critical for developing targeted interventions that can effectively disrupt this cycle and restore normal muscle function. This review aims to provide a comprehensive analysis of the acute molecular response following ACL injury, focusing on the key inflammatory mediators, nociceptive signaling pathways, and neuromuscular changes that underlie AMI. By examining the temporal dynamics of cytokine expression and their interactions with motor control circuits, we hope to elucidate the complex pathophysiology of ACL injuries and highlight potential therapeutic targets for improving recovery outcomes. Furthermore, we explore how these molecular alterations influence rehabilitation strategies, with a particular emphasis on personalized approaches that consider individual variations in inflammatory and neuromuscular responses, ultimately aiming to optimize functional recovery and reduce the risk of long-term complications.

Molecular and Neuromuscular Changes Post ACL Injury

The molecular and neuromuscular changes that occur following an ACL injury are the result of a highly orchestrated and multifaceted response that involves inflammatory, neurochemical, and structural adaptations, all of which culminate in significant motor dysfunction. The injury initiates a cascade of immediate molecular events within the joint environment, characterized by the release of damage-associated molecular patterns (DAMPs) such as high-mobility group box 1 (HMGB1), heat shock proteins (HSPs), and mitochondrial DNA (mtDNA) from ruptured cells. These DAMPs bind to pattern recognition receptors (PRRs), including Toll-like receptors (TLRs) and NOD-like receptors (NLRs), expressed on resident synoviocytes, chondrocytes, and infiltrating immune cells. This binding activates a series of intracellular signaling pathways, such as the NF-κB, MAPK, and JAK/STAT pathways, leading to the rapid upregulation of pro-inflammatory cytokines like interleukin-1 beta (IL-1β), IL-6, TNF-α, and IL-8. These cytokines amplify the inflammatory response by promoting the recruitment of neutrophils and macrophages to the synovial fluid, where they release a secondary wave of inflammatory mediators, including reactive oxygen species (ROS), proteolytic enzymes like matrix metalloproteinases (MMPs), and catabolic factors that degrade the

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extracellular matrix (ECM) and contribute to tissue damage. The elevated levels of IL-1β and TNF-α also sensitize peripheral nociceptors by upregulating pro-nociceptive ion channels such as transient receptor potential vanilloid 1 (TRPV1) and acid-sensing ion channels (ASICs) on sensory neurons, lowering their activation thresholds and leading to heightened pain sensitivity—a phenomenon known as peripheral sensitization. This nociceptive input is then transmitted through group III and IV afferent fibers to the spinal cord's dorsal horn, where the sustained release of excitatory neurotransmitters, including glutamate, substance P, and calcitonin gene-related peptide (CGRP), induces central sensitization by activating NMDA receptors and upregulating brain-derived neurotrophic factor (BDNF) expression. BDNF binds to TrkB receptors on dorsal horn neurons, altering chloride homeostasis by downregulating the potassium-chloride cotransporter KCC2, which shifts GABAergic and glycinergic inhibition from hyperpolarization to depolarization, resulting in disinhibition of spinal nociceptive circuits and prolonged motor neuron inhibition. These spinal changes are mirrored by supraspinal adaptations, where increased activity in the primary motor cortex (M1), prefrontal cortex (PFC), and supplementary motor area (SMA) reflects maladaptive reorganization in response to impaired proprioceptive input and altered sensory processing. The chronic presence of pro-inflammatory cytokines and neurotrophic factors like nerve growth factor (NGF) further promotes structural changes in cortical and subcortical networks, reinforcing the inhibitory effects on motor control and leading to a state of Arthrogenic Muscle Inhibition (AMI). AMI manifests as a marked reduction in voluntary muscle activation, particularly in the quadriceps, which is critical for knee stabilization and functional recovery. This neuromuscular inhibition is driven not only by altered peripheral feedback but also by central mechanisms, including increased inhibitory neurotransmission at the spinal level and disrupted corticospinal excitability, resulting in diminished motor unit recruitment, decreased force production, and impaired muscle coordination. The net effect is a state of neuromuscular dysfunction that complicates rehabilitation and prolongs recovery, as the molecular drivers of inflammation, nociception, and neural inhibition create a self-perpetuating cycle that must be addressed through targeted therapeutic strategies aimed at restoring both molecular and neuromuscular balance (Table 1).

Table 1. The table details the complex molecular and neuromuscular changes following an ACL injury and highlights targeted rehabilitation strategies to optimize recovery. It covers the acute inflammatory response, nociceptive sensitization, cytokine-mediated muscle atrophy, and cortical reorganization, explaining how these factors disrupt motor control and contribute to Arthrogenic Muscle Inhibition (AMI). Rehabilitation interventions, such as Blood Flow Restriction (BFR) therapy, resistance training, and specialized nutritional strategies, are recommended to counteract muscle atrophy, reduce inflammation, and restore neuromuscular function. Additionally, dietary modifications incorporating anti-inflammatory and antioxidant-rich foods, along with the use of procyanidins, are suggested to support tissue repair and minimize maladaptive plasticity.

Molecular/ Cellular Event	Description	Key Mediators/ Pathways	Impact on Joint and Neuromuscular Function	Specific Rehabilitation Strategies and Interventions
1. Rapid Joint Swelling and Intra-articular Bleeding	The immediate response involves joint effusion, hemarthrosis, and an acute pro-inflammatory cascade, setting the stage for tissue damage and neuromuscular inhibition.	DAMPs (HMGB1, mtDNA), TLRs (TLR4), IL-1, IL-6, TNF-α, MMPs, ROS	- DAMPs activate TLR4 receptors on synoviocytes and macrophages, initiating the NF-κB pathway, resulting in the release of IL-1, IL-6, and TNF-α. - MMPs degrade ECM components (collagen, aggrecan), weakening joint structures and	Early-phase Interventions: - Cryotherapy: Reduces intra-articular pressure and blood flow, limiting hemarthrosis. - NSAIDs and COX-2 inhibitors: Decrease prostaglandin synthesis to reduce pain and swelling.

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			increasing inflammatory signaling. - ROS damage synovial tissue and perpetuate the inflammatory loop, leading to hemarthrosis and pain sensitization. - Direct activation of nociceptors (TRPV1, ASICs) by hemoglobin breakdown products (heme, iron ions), inducing AMI.	- Aspiration of joint effusion: Lowers intra-articular pressure, alleviating pain and improving range of motion (ROM). - Neuromuscular Electrical Stimulation (NMES): Prevents quadriceps atrophy by promoting early muscle activation. - Blood Flow Restriction (BFR) Training: Prevents muscle atrophy by maintaining muscle hypertrophy and strength with low-load exercises, reducing strain on the joint.
2. Nociceptor Sensitization and Central Sensitization	Persistent nociceptive signaling from joint inflammation leads to spinal and supraspinal sensitization, reinforcing AMI and altering motor control.	Substance P, CGRP, IL-1β, TNF-α, Glutamate, NMDA Receptors, BDNF	- Substance P and CGRP released from nociceptors enhance NMDA receptor activation, increasing calcium influx and excitatory signaling. - Elevated IL-1β and TNF-α activate spinal microglia, producing additional pro-nociceptive mediators (ROS, ATP). - Central sensitization leads to GABAergic and glycinergic disinhibition, increasing excitability of nociceptive pathways, and amplifying pain signals.	Pain Management and Neural Modulation: - TENS (Transcutaneous Electrical Nerve Stimulation): Reduces pain by modulating spinal and supraspinal pathways. - Motor Imagery and Mirror Therapy: Helps re-establish cortical patterns disrupted by pain and sensory loss. - Pharmacological Interventions: Gabapentin or NMDA receptor antagonists to reduce central sensitization. - Graded Motor Exposure: Gradual increase in activity to desensitize central pain pathways. - Blood Flow Restriction (BFR) Therapy: Utilizes low-pressure BFR to desensitize pain pathways while

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				maintaining muscle mass and strength.
3. Cytokine-Mediated Muscle Atrophy	Pro-inflammatory cytokines enhance proteolysis and suppress protein synthesis, promoting muscle catabolism and atrophy.	IL-6, TNF-α, FoxO, MuRF1, Atrogin-1, mTOR Inhibition	- IL-6 activates STAT3, leading to increased expression of MuRF1 and Atrogin-1 through FoxO transcription factors. - TNF-α and IL-1β inhibit mTOR signaling, suppressing muscle protein synthesis and promoting muscle wasting. - This results in significant quadriceps muscle atrophy, impairing knee stability and increasing reinjury risk.	Resistance Training and Anti-catabolic Strategies: - Early Low-Load Resistance Isometric/ Eccentric Exercise: Prevents atrophy and preserves muscle strength. - NMES: Activates muscle fibers to maintain muscle size and improve recruitment patterns. - Blood Flow Restriction (BFR) Training: Allows hypertrophic and strength gains with low mechanical loads, reducing joint stress. - Nutritional Support (High-Protein Diet): Incorporate high-quality proteins (e.g., lean meats, fish, plant-based proteins) to support muscle protein synthesis and reduce catabolic effects. - Omega-3 Fatty Acids: Reduce inflammation and preserve muscle mass by modulating IL-1β and TNF-α signaling.
4. Inflammatory-Induced Mitochondrial Dysfunction	Chronic inflammation disrupts mitochondrial function, leading to reduced ATP production, oxidative stress, and muscle fatigue.	IL-6, IL-8, ROS, FoxO, AMPK, mtDNA Damage	- IL-6 and IL-8 increase ROS production, inducing mitochondrial permeability transition pore (mPTP) opening, leading to mtDNA damage. - Reduced ATP production affects muscle endurance, compromising contractile function and rehabilitation progress. - ROS-induced FoxO	Rehabilitation Strategies to Restore Mitochondrial Health: - Aerobic Exercise Training: Enhances mitochondrial biogenesis and improves oxidative capacity. - High-Intensity Interval Training (HIIT): Stimulates mitochondrial adaptation and increases ATP production.

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			activation triggers mitochondrial autophagy (mitophagy), contributing to further muscle degradation.	- Antioxidant Supplementation: Procyanidins, resveratrol, or NAC to reduce ROS and enhance mitochondrial function. - Nutritional Support (High Antioxidant Diet): Incorporate berries, green tea, and dark chocolate to boost endogenous antioxidant levels and support mitochondrial function.
5. Cortical Reorganization and Maladaptive Plasticity	Disrupted sensory feedback leads to compensatory cortical reorganization, impairing motor coordination and increasing risk of reinjury.	BDNF, Synapsin I, PSD-95, c-Fos, NGF	- Elevated BDNF promotes maladaptive cortical plasticity in the M1, PFC, and SMA. - Increased PSD-95 and synapsin I levels indicate aberrant synaptic remodeling, disrupting sensorimotor integration. - Excessive cortical involvement in motor control leads to inefficient and compensatory movement patterns.	Motor Retraining and Cortical Rehabilitation: - Neurofeedback and Motor Imagery: Rewires cortical circuits by reinforcing correct movement patterns. - Task-specific Training: Focuses on re-establishing automatic and reflexive motor control. - Proprioceptive Exercises: Enhances sensory feedback and reduces reliance on higher cortical centers for movement control. - Cognitive-Motor Training: Combines mental and physical exercises to promote balanced cortical engagement (e.g Stroboscopic Glasses). - Blood Flow Restriction (BFR) Therapy: Combined with motor retraining to enhance neuromuscular adaptation without increasing joint stress.

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6. Anti-inflammatory Shift (4 Weeks Post-Surgery)	Transition from pro-inflammatory to anti-inflammatory cytokine environment supports tissue repair and resolution of inflammation.	IL-10, IL-4, TGF-β, SOCS3, miR-146a	- IL-10 activates STAT3, promoting M2 macrophage polarization and reducing pro-inflammatory gene expression (SOCS3, miR-146a). - IL-4 enhances ECM remodeling by upregulating arginase-1 and collagen synthesis pathways. - TGF-β promotes tissue repair but may induce fibrosis if dysregulated, leading to joint stiffness and scar formation.	Promoting Anti-inflammatory Environment and Tissue Repair: - Manual Therapy: Reduces fibrosis by promoting healthy collagen alignment and preventing scar tissue formation. - Progressive ROM Exercises: Encourages proper tissue healing and prevents adhesions. - IL-10 Enhancers: Use of bioactive compounds like curcumin to promote IL-10 signaling. - High Omega-3 Fatty Acid Intake: Modulates inflammatory response and promotes anti-inflammatory signaling pathways. - Antifibrotic Strategies: Monitor collagen deposition with imaging and adjust exercise protocols to prevent excess scar formation.
7. Influence of Dietary Polyphenols (Procyanidins)	Procyanidins modulate inflammatory pathways, central sensitization, and oxidative stress, aiding neuromuscular recovery.	ROS Scavenging, MAPK Inhibition, KCC2 Upregulation, TRPV1 Modulation	- Procyanidins reduce IL-1β, TNF-α, and COX-2 expression by inhibiting MAPK and NF-κB pathways. - Restoration of GABAergic inhibition via KCC2 upregulation mitigates AMI. - TRPV1 channel inhibition reduces nociceptive neurotransmitter release (Substance P, CGRP), decreasing pain.	Incorporating Procyanidins in Rehabilitation: - Dietary Supplementation: Include procyanidin-rich foods (grape seed extract, apples, cocoa) to enhance anti-inflammatory effects. - Adjunct Therapy: Combine with physical therapy to maximize pain reduction and functional recovery. - Nutritional Support (High Polyphenol Diet): Include green tea, dark berries, and dark chocolate to

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		support recovery and reduce oxidative stress. - Prolonged Use: Long-term incorporation of polyphenols to maintain anti-inflammatory effects and prevent maladaptive plasticity.

1. Rapid Joint Swelling and Intra-Articular Bleeding

The initial response following an ACL tear involves a rapid onset of joint effusion and intra-articular bleeding, which generates a cascade of molecular events that significantly disrupt motor control by inducing neuromuscular inhibition through various biochemical pathways. The mechanical disruption caused by the injury leads to the release of damage-associated molecular patterns (DAMPs) from ruptured cells, which activate pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs) on resident synoviocytes and macrophages within the joint. This activation triggers a robust pro-inflammatory response, resulting in the release of cytokines, including interleukin-1 (IL-1), IL-6, and tumor necrosis factor-alpha (TNF-α), which rapidly amplify the local inflammatory environment. These cytokines and chemokines recruit neutrophils and macrophages into the synovial space, which further produce reactive oxygen species (ROS) and proteolytic enzymes such as matrix metalloproteinases (MMPs), exacerbating cartilage degradation and synovial irritation. The influx of inflammatory mediators into the joint fluid leads to alterations in synovial viscosity and pH, creating a hostile biochemical milieu that stimulates nociceptive neurons and sensitizes afferent fibers.

The presence of blood and plasma proteins in the synovial fluid induces hemarthrosis, which acts as a potent irritant, increasing intra-articular pressure and directly stimulating nociceptors on synovial and capsular tissues. Hemoglobin breakdown products, such as heme and iron ions, catalyze further oxidative reactions, contributing to lipid peroxidation and the formation of advanced glycation end-products (AGEs) that sustain chronic inflammation. This environment not only provokes a sustained release of pro-inflammatory cytokines but also triggers the activation of endothelial cells lining the joint capsule, promoting vascular permeability and perpetuating synovial swelling. The molecular interactions between IL-1 and TNF-α and their receptors (IL-1R and TNFR) on the surface of neurons and myocytes initiate signaling cascades that involve nuclear factor-kappa B (NF-κB) and mitogen-activated protein kinases (MAPKs), leading to upregulation of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2). These enzymes produce nitric oxide (NO) and prostaglandins, respectively, which further enhance local inflammation and nociception.

As a consequence, nociceptive afferent groups III and IV, which innervate the joint capsule and surrounding musculature, become hyperexcitable, transmitting persistent pain signals to the spinal cord. This ongoing nociceptive barrage initiates a phenomenon known as central sensitization, where sustained input from injured tissue leads to enhanced synaptic efficacy at the spinal cord level, mediated by increased expression of excitatory neurotransmitters such as glutamate and substance P. Concomitantly, inhibitory neurotransmitters, such as gamma-aminobutyric acid (GABA) and glycine, are downregulated, causing a shift in the balance of excitatory-inhibitory synaptic activity, ultimately leading to decreased inhibition of nociceptive signals. This imbalance in spinal neurotransmission results in widespread inhibitory effects on alpha motor neurons, particularly those innervating the vastus medialis oblique (VMO), leading to the development of AMI.

The molecular alterations at the spinal level are mirrored by supraspinal changes, as seen in the corticospinal excitability changes identified by Rice et al. (2004). These changes likely result from disrupted sensory feedback to the primary motor cortex (M1), leading to maladaptive cortical

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reorganization and altered interhemispheric communication. Neuroimaging studies have shown that ACL injuries lead to increased activation of the prefrontal cortex (PFC) and supplementary motor areas (SMA), indicative of compensatory strategies to overcome impaired motor control. The disrupted proprioceptive feedback from the knee joint may lead to decreased gamma motor neuron drive, which in turn reduces muscle spindle sensitivity, diminishing the muscle's ability to respond to changes in joint position. On a molecular level, this maladaptive reorganization is associated with altered expression of brain-derived neurotrophic factor (BDNF) and changes in synaptic plasticity markers, such as synapsin I and postsynaptic density protein 95 (PSD-95), which are critical for maintaining normal sensorimotor integration and voluntary muscle activation.

The prolonged presence of inflammatory mediators like IL-1 and TNF-α within the joint fluid also impacts satellite cell activity in the surrounding muscles, impairing their ability to proliferate and differentiate in response to injury. This disruption of satellite cell function is mediated through the inhibition of the myogenic regulatory factors MyoD and myogenin, which are essential for muscle repair and regeneration. Moreover, chronic inflammation induces the expression of transforming growth factor-beta (TGF-β), a cytokine that promotes fibrosis by upregulating connective tissue growth factor (CTGF) and increasing extracellular matrix (ECM) deposition. This fibrotic response can further compromise muscle extensibility and joint range of motion, creating a vicious cycle where impaired muscle function exacerbates joint instability, leading to recurrent inflammation and perpetuation of AMI.

At the molecular level, the presence of inflammatory cytokines like IL-6 and IL-8 also influences mitochondrial function within muscle fibers, promoting oxidative stress and the accumulation of mitochondrial DNA (mtDNA) damage. This mitochondrial dysfunction leads to a decline in adenosine triphosphate (ATP) production, reducing the energy available for sustained muscle contractions. The metabolic stress induced by cytokine signaling can trigger the activation of the ubiquitin-proteasome system (UPS), leading to increased muscle protein degradation and atrophy, particularly in the quadriceps. This process is regulated by the transcription factor Forkhead box O (FoxO), which upregulates muscle-specific E3 ubiquitin ligases, such as muscle atrophy F-box (MAFbx) and muscle ring-finger protein-1 (MuRF1), further contributing to the loss of muscle mass and function observed post-ACL injury.

2. Acute Inflammatory Response: Key Cytokines and Chemokines

The acute inflammatory response following an ACL injury is characterized by a complex and rapid activation of molecular signaling pathways involving a diverse array of pro-inflammatory cytokines, chemokines, and damage-associated molecular patterns (DAMPs), which converge to create a highly reactive biochemical environment within the synovial fluid. Immediately after the injury, the disruption of the extracellular matrix (ECM) and the rupture of local cells, including chondrocytes, synoviocytes, and endothelial cells, result in the release of intracellular components such as high-mobility group box 1 (HMGB1), ATP, and heat shock proteins (HSPs), which serve as potent DAMPs. These molecules bind to Toll-like receptors (TLRs) and receptor for advanced glycation end-products (RAGE) on synovial macrophages and fibroblasts, triggering the rapid activation of intracellular signaling cascades, including the mitogen-activated protein kinase (MAPK) and Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathways. This leads to the transcriptional upregulation of key inflammatory cytokines such as interleukin-1 (IL-1), IL-6, tumor necrosis factor-alpha (TNF-α), and IL-8, which amplify the inflammatory response by promoting a paracrine signaling loop that recruits additional immune cells to the site of injury.

Interleukin-1 (IL-1) and IL-1β are pivotal cytokines in this acute inflammatory milieu, exerting their effects primarily through the activation of the IL-1 receptor type I (IL-1R1), which is expressed on synovial fibroblasts, chondrocytes, and endothelial cells. The binding of IL-1 to IL-1R1 activates downstream adaptor proteins such as MyD88 (myeloid differentiation primary response 88) and IRAK4 (interleukin-1 receptor-associated kinase 4), which phosphorylate and activate TRAF6 (tumor necrosis factor receptor-associated factor 6). TRAF6 then mediates the activation of the IκB kinase (IKK) complex, leading to the nuclear translocation of nuclear factor-kappa B (NF-κB), a master

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transcriptional regulator of inflammatory genes. Within the nucleus, NF-κB binds to promoter regions of pro-inflammatory genes, including those encoding matrix metalloproteinases (MMPs), inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), and additional cytokines such as IL-6 and TNF-α. MMPs, particularly MMP-1 and MMP-3, degrade collagen and aggrecan within the cartilage matrix, leading to structural damage and increasing the release of cartilage breakdown products that further stimulate synovial inflammation. IL-1 also promotes the expression of aggrecanases, such as ADAMTS-4 and ADAMTS-5, which cleave proteoglycans and reduce the compressive strength of the cartilage, accelerating joint degeneration and perpetuating the inflammatory state.

Simultaneously, the elevation of IL-6 levels in the synovial fluid initiates a distinct but interrelated signaling pathway through its binding to the IL-6 receptor (IL-6R) and subsequent interaction with glycoprotein 130 (gp130). This interaction activates the JAK/STAT pathway, leading to the phosphorylation and dimerization of STAT3, which translocates into the nucleus to regulate the transcription of genes involved in acute-phase responses and muscle catabolism. IL-6 is known to upregulate the expression of muscle-specific E3 ubiquitin ligases, such as muscle RING finger 1 (MuRF1) and atrogin-1, through the activation of the transcription factor Forkhead box O (FoxO), resulting in enhanced proteolysis and muscle atrophy. The catabolic effects of IL-6 are further potentiated by its ability to activate AMP-activated protein kinase (AMPK) in muscle cells, which suppresses the mammalian target of rapamycin (mTOR) pathway, a key regulator of protein synthesis. This dual action of IL-6 on both protein degradation and inhibition of protein synthesis leads to a net loss of muscle mass, particularly in the quadriceps, which is crucial for knee stabilization and function.

Interleukin-8 (IL-8), a potent chemokine produced by activated macrophages, fibroblasts, and endothelial cells in response to IL-1 and TNF-α, plays a critical role in recruiting neutrophils and other immune cells to the injured joint. IL-8 exerts its effects primarily through binding to its G-protein-coupled receptors, CXCR1 and CXCR2, on neutrophils, which triggers intracellular signaling cascades involving phospholipase C (PLC), protein kinase C (PKC), and the MAPK pathway. This signaling cascade results in the production of ROS and the release of neutrophil extracellular traps (NETs), which contain antimicrobial proteins and DNA, contributing to further joint tissue damage. The ROS generated by neutrophils oxidize lipids, proteins, and DNA, creating a self-perpetuating cycle of oxidative stress and inflammation. Moreover, the interaction of IL-8 with its receptors on endothelial cells promotes the expression of adhesion molecules, such as E-selectin, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1), which facilitate the transendothelial migration of additional leukocytes into the synovium, further sustaining the inflammatory response.

The presence of TNF-α in the synovial fluid exacerbates these effects by binding to its receptors, TNFR1 and TNFR2, on various cell types, including synoviocytes and myocytes, initiating apoptotic and necrotic cell death pathways via the recruitment of TRADD (TNFR-associated death domain) and FADD (Fas-associated death domain) adaptor proteins. This results in the activation of caspases, particularly caspase-8 and caspase-3, which degrade cytoskeletal and nuclear proteins, leading to cell death and the release of additional DAMPs that sustain the inflammatory cycle. Furthermore, TNF-α activates NF-κB and MAPK pathways, promoting the transcription of pro-inflammatory genes and enhancing the expression of pro-apoptotic proteins such as Bax, while simultaneously inhibiting anti-apoptotic proteins like Bcl-2, thereby tipping the balance towards cell death. This extensive cellular damage and the sustained release of pro-inflammatory mediators contribute to an environment of chronic inflammation, joint tissue degradation, and persistent nociceptive activation, creating a biochemical state that disrupts normal muscle activation and significantly impairs motor control.

3. Anti-inflammatory Shift: Four Weeks Post-Surgery

During the post-surgical recovery phase following ACL injury, the synovial microenvironment undergoes a tightly regulated molecular transition characterized by a reduction in pro-inflammatory signaling and a corresponding increase in anti-inflammatory mediators that facilitate tissue repair

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and regeneration. This shift is primarily driven by the orchestrated interplay between specific anti-inflammatory cytokines, including interleukin-10 (IL-10) and interleukin-4 (IL-4), which modulate a broad range of intracellular signaling pathways to suppress inflammation, promote cellular proliferation, and drive tissue remodeling. IL-10, secreted predominantly by regulatory T cells (Tregs), M2 macrophages, and other immune regulatory cells, exerts its effects through binding to the IL-10 receptor (IL-10R), a complex consisting of IL-10R1 and IL-10R2 subunits. Upon ligand binding, IL-10R initiates the recruitment and activation of Janus kinases (JAK1 and TYK2), leading to the phosphorylation of the transcription factor STAT3. Phosphorylated STAT3 forms homodimers that translocate into the nucleus, where they bind to specific promoter regions of anti-inflammatory genes, upregulating the expression of suppressor of cytokine signaling (SOCS) proteins, particularly SOCS3. SOCS3 acts as a negative regulator of cytokine signaling by inhibiting JAK activity and blocking further signal transduction through the NF-κB and MAPK pathways. This inhibition of NF-κB, a key pro-inflammatory transcription factor, results in a broad suppression of inflammatory gene expression, including that of IL-1β, IL-6, TNF-α, and various chemokines such as CCL2 and CXCL8, thereby reducing the recruitment of pro-inflammatory immune cells such as neutrophils and M1 macrophages to the site of injury.

In addition to its role in suppressing inflammation, IL-10 enhances tissue repair by modulating macrophage function, promoting their polarization from a pro-inflammatory M1 phenotype to a reparative M2 phenotype. M2 macrophages secrete high levels of transforming growth factor-beta (TGF-β), a pleiotropic cytokine that binds to its receptor complex (TGF-βRI and TGF-βRII) on synovial fibroblasts and chondrocytes, initiating the phosphorylation of receptor-regulated Smad proteins (Smad2 and Smad3). These phosphorylated Smads form complexes with the common mediator Smad4, which translocate to the nucleus and regulate the transcription of genes involved in extracellular matrix (ECM) synthesis, including collagen type I (COL1A1), collagen type III (COL3A1), and fibronectin. TGF-β also induces the expression of connective tissue growth factor (CTGF), which enhances fibroblast proliferation and promotes the deposition of ECM components, thereby stabilizing the joint structure and supporting the integrity of the synovial membrane. However, the overactivation of TGF-β signaling can lead to excessive collagen deposition and fibrosis, underscoring the need for a precise balance between its anabolic and catabolic effects to ensure optimal tissue repair without pathological scarring.

Concurrently, IL-4, a cytokine primarily produced by Th2 cells, eosinophils, and mast cells, contributes to the resolution of inflammation and tissue healing by acting through the IL-4 receptor complex (IL-4Rα/γc) on target cells. The engagement of IL-4Rα initiates the activation of STAT6, which drives the transcriptional program associated with M2 macrophage polarization, including upregulation of genes such as ARG1 (arginase 1), which competes with inducible nitric oxide synthase (iNOS) for the substrate L-arginine, reducing the production of pro-inflammatory nitric oxide (NO) and increasing the production of polyamines and proline, which are critical for cell proliferation and collagen synthesis. IL-4 also upregulates mannose receptor (CD206) and chitinase-like proteins, which contribute to the clearance of debris and facilitate tissue remodeling. Furthermore, IL-4 enhances the anti-inflammatory environment by inducing the secretion of IL-13, another Th2-associated cytokine that acts synergistically with IL-4 to promote the resolution of inflammation and stimulate epithelial cell proliferation and repair. The combined action of IL-4 and IL-13 results in the activation of STAT6 in fibroblasts, driving the expression of ECM components such as decorin and biglycan, which are essential for collagen fibrillogenesis and tissue tensile strength.

Moreover, IL-4 and IL-10 have direct effects on synovial fibroblasts and chondrocytes, reducing the expression of catabolic enzymes such as matrix metalloproteinase-13 (MMP-13) and aggrecanases (ADAMTS-4 and ADAMTS-5), which are typically upregulated during the acute inflammatory phase and contribute to cartilage breakdown. By inhibiting these catabolic pathways, IL-4 and IL-10 preserve cartilage integrity and promote the synthesis of proteoglycans and glycosaminoglycans, which restore the biomechanical properties of the joint. Additionally, IL-10 enhances the expression of tissue inhibitors of metalloproteinases (TIMPs), which counterbalance MMP activity and prevent

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excessive matrix degradation. This modulation of ECM turnover is critical for maintaining the structural and functional stability of the joint during the reparative phase.

The anti-inflammatory shift is also regulated at the epigenetic level by microRNAs (miRNAs) and histone modifications that fine-tune cytokine gene expression. For example, miR-146a, an IL-10-induced microRNA, targets TRAF6 and IRAK1, key components of the IL-1 and TLR signaling pathways, thereby providing an additional layer of inhibition on NF-κB activation. Furthermore, IL-4 induces the expression of miR-223, which modulates macrophage polarization by targeting the M1-associated transcription factor PU.1, promoting a shift toward an M2 phenotype. Histone acetylation and methylation patterns, regulated by enzymes such as histone acetyltransferases (HATs) and histone deacetylases (HDACs), also play a role in the transcriptional regulation of cytokine genes, ensuring that the expression of pro-inflammatory mediators is suppressed while anti-inflammatory genes are upregulated. This epigenetic regulation, combined with the precise control of cytokine signaling pathways, ensures a balanced transition from inflammation to tissue repair, preventing chronic inflammation and fibrosis that could otherwise lead to joint stiffness and impaired mobility.

In summary, the anti-inflammatory shift during the post-surgical recovery phase is governed by a complex network of cytokine signaling pathways, intracellular transcriptional regulators, and epigenetic modifications that collectively resolve the acute inflammatory response, promote tissue repair, and restore joint function. This tightly regulated molecular environment is essential for achieving a functional recovery and minimizing long-term complications such as fibrosis, muscle atrophy, and impaired motor control.

4. Nociception and Pain: Impact on Motor Control

Nociceptive inputs originating from the injured ACL significantly contribute to the development and persistence of Arthrogenic Muscle Inhibition (AMI) through a complex array of molecular mechanisms that involve the activation and sensitization of both peripheral and central nociceptive pathways. The key players in this process are group III and IV afferent fibers, which are highly responsive to mechanical, thermal, and chemical stimuli released from the damaged tissue. These afferent fibers transmit noxious signals from the injured knee joint to the spinal cord's dorsal horn, where they synapse with second-order neurons and release a variety of excitatory neurotransmitters, including glutamate, substance P, and calcitonin gene-related peptide (CGRP). The release of substance P, acting through the neurokinin-1 (NK1) receptor, and CGRP via its CGRP receptors, initiates intracellular signaling cascades such as protein kinase C (PKC) and mitogen-activated protein kinase (MAPK) pathways, leading to increased phosphorylation of NMDA (N-methyl-D-aspartate) receptors on spinal neurons. This phosphorylation enhances calcium influx and depolarizes the postsynaptic membrane, establishing a phenomenon known as central sensitization, in which spinal neurons become hyperresponsive to subsequent stimuli. Central sensitization is further characterized by an increased expression of voltage-gated sodium channels (Nav1.7 and Nav1.8) and transient receptor potential (TRP) channels on nociceptive neurons, which amplify their excitability and contribute to prolonged inhibition of the quadriceps muscle, particularly the vastus medialis oblique (VMO).

The activation of these nociceptive pathways also triggers the release of pro-inflammatory cytokines, such as interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α), from activated spinal astrocytes and microglia. These glial cells become reactive and undergo morphological and functional changes, producing high levels of pro-inflammatory mediators that contribute to the maintenance of a pro-nociceptive environment. IL-1β and TNF-α activate the NF-κB signaling pathway, leading to the transcriptional upregulation of cyclooxygenase-2 (COX-2) and the subsequent production of prostaglandin E2 (PGE2), which further sensitizes nociceptive neurons by binding to EP receptors and increasing intracellular cAMP levels. Additionally, reactive astrocytes release glutamate and ATP, which activate purinergic receptors such as P2X4 and P2X7 on microglia, triggering the release of brain-derived neurotrophic factor (BDNF). BDNF binds to the TrkB receptor on dorsal horn neurons, altering chloride homeostasis by downregulating the expression of the potassium-chloride cotransporter KCC2, thereby shifting the GABAergic inhibitory responses from

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hyperpolarization to depolarization. This results in a loss of inhibitory tone and further enhances the excitability of nociceptive pathways, prolonging pain and contributing to the chronic inhibition of motor neurons innervating the quadriceps.

The persistent activation of nociceptive pathways not only modulates spinal circuits but also has significant effects at supraspinal levels. Functional neuroimaging studies by Grooms et al. (2017) have shown that ACL-injured individuals exhibit global increases in activity within the prefrontal cortex (PFC), sensorimotor cortex, and supplementary motor areas (SMA), indicative of compensatory mechanisms aimed at overcoming the diminished proprioceptive input from the injured joint. At the molecular level, this hyperactivity is driven by increased expression of synaptic plasticity-related proteins, including synapsin I, postsynaptic density protein 95 (PSD-95), and the immediate early gene c-fos. These changes are further reinforced by elevated levels of neurotrophins such as nerve growth factor (NGF) and BDNF, which promote synaptogenesis and dendritic growth. While this neuroplasticity initially serves as a compensatory mechanism, prolonged alterations in cortical excitability can lead to maladaptive plasticity, resulting in inefficient motor patterns and an increased reliance on higher-order cognitive strategies for motor control.

Interestingly, recent research has indicated that specific dietary polyphenols, such as procyanidins, may modulate these nociceptive and inflammatory processes through their antioxidant and anti-inflammatory properties. Procyanidins, which are oligomeric flavonoids found in high concentrations in grape seeds, apples, and cocoa, have been shown to attenuate neuroinflammation by targeting key signaling pathways involved in central sensitization and glial activation. Procyanidins exert their effects by scavenging reactive oxygen species (ROS) and inhibiting the activation of NADPH oxidase, thereby reducing oxidative stress within the spinal cord. This reduction in oxidative stress prevents the activation of MAPK and NF-κB pathways in microglia and astrocytes, leading to decreased production of IL-1β, TNF-α, and PGE2. Additionally, procyanidins have been found to inhibit the phosphorylation of extracellular signal-regulated kinases (ERK1/2) and p38 MAPK in nociceptive neurons, thereby reducing the expression of pro-nociceptive mediators such as COX-2 and inducible nitric oxide synthase (iNOS). This inhibition of inflammatory pathways not only attenuates central sensitization but also restores GABAergic inhibition by upregulating KCC2 expression, reversing the chloride ion imbalance induced by BDNF-TrkB signaling.

Procyanidins also modulate the activity of the transient receptor potential vanilloid 1 (TRPV1) channel, a key mediator of thermal and inflammatory pain, by inhibiting its phosphorylation and reducing calcium influx in nociceptive neurons. This leads to a decrease in TRPV1-mediated release of CGRP and substance P, further dampening nociceptive transmission at the spinal level. Moreover, procyanidins have been shown to promote the polarization of macrophages and microglia toward an anti-inflammatory M2 phenotype, characterized by increased production of IL-10 and TGF-β, which contribute to tissue repair and resolution of inflammation. By modulating these molecular pathways, procyanidins not only reduce the excitability of nociceptive pathways but also enhance the anti-inflammatory cytokine environment, potentially mitigating AMI and facilitating the restoration of normal motor control.

The presence of procyanidins in the post-ACL injury setting could therefore represent a novel therapeutic approach for targeting both peripheral and central mechanisms underlying AMI. Their ability to modulate key molecular targets—ranging from ROS production and pro-inflammatory cytokine expression to the regulation of synaptic plasticity proteins and ion channels—makes them a promising candidate for attenuating chronic pain and reversing the maladaptive plasticity associated with prolonged nociceptive input. Through these multifaceted molecular interactions, procyanidins may provide a complementary strategy to existing rehabilitation approaches, promoting faster recovery of neuromuscular function and reducing the risk of long-term complications such as muscle atrophy and reinjury.

Conclusion

ACL injuries initiate a cascade of highly coordinated molecular and neuromuscular changes that unfold in distinct temporal stages, each governed by specific cellular events, cytokine profiles, and

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neuromuscular adaptations that collectively disrupt voluntary muscle activation and motor control. Upon ligament rupture, the immediate mechanical insult leads to the release of damage-associated molecular patterns (DAMPs), such as high mobility group box 1 (HMGB1), ATP, and heat shock proteins (HSPs), which bind to pattern recognition receptors (PRRs) like Toll-like receptors (TLRs) and NOD-like receptors (NLRs) on synovial cells and resident immune cells. This interaction triggers the rapid activation of intracellular signaling cascades, including the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and mitogen-activated protein kinase (MAPK) pathways, culminating in the production and secretion of pro-inflammatory cytokines such as interleukin-1 beta (IL-1β), IL-6, and tumor necrosis factor-alpha (TNF-α). These cytokines initiate an acute inflammatory response, which serves to recruit neutrophils and macrophages into the synovial fluid and surrounding tissues. Neutrophils, in particular, release reactive oxygen species (ROS) and proteolytic enzymes like matrix metalloproteinases (MMPs), which further degrade extracellular matrix (ECM) components, exacerbating tissue damage and prolonging the inflammatory phase. The elevated ROS levels also activate redox-sensitive transcription factors such as activator protein 1 (AP-1) and NF-κB, amplifying the inflammatory cascade and creating a self-sustaining loop that drives continued cytokine production and joint degeneration.

The presence of these pro-inflammatory cytokines has profound effects on both the joint environment and the neuromuscular system. IL-1β and TNF-α, for instance, sensitize peripheral nociceptors by upregulating the expression of pro-nociceptive ion channels such as transient receptor potential vanilloid 1 (TRPV1) and acid-sensing ion channels (ASICs) on sensory neurons, thereby lowering their activation thresholds and leading to heightened pain sensitivity. This increased sensitivity contributes to the phenomenon of peripheral sensitization, where previously innocuous stimuli become capable of triggering nociceptive responses. Concurrently, IL-6 acts through its receptor complex (IL-6R and gp130) to activate the JAK/STAT signaling pathway in both immune cells and muscle fibers, resulting in the transcription of catabolic genes that promote muscle protein degradation through the ubiquitin-proteasome system (UPS) and the autophagy-lysosome pathway. This catabolic state is marked by increased expression of muscle-specific E3 ubiquitin ligases, such as muscle RING finger-1 (MuRF1) and atrogin-1, which target structural muscle proteins for degradation, leading to significant muscle atrophy and weakness. The resulting loss of muscle mass, particularly in the quadriceps group, severely impairs voluntary muscle activation, reducing the joint's ability to stabilize during movement and increasing the risk of further injury.

As the inflammatory response progresses, nociceptive signals originating from the injured joint propagate through the dorsal horn of the spinal cord and ascend to higher cortical centers, where they contribute to both spinal and supraspinal plasticity. In the spinal cord, the activation of microglia and astrocytes by cytokines such as IL-1β and TNF-α results in the release of additional pro-inflammatory mediators and neuroactive substances, including glutamate and ATP, which activate purinergic receptors such as P2X4 and P2X7 on microglia. This activation induces the release of brain-derived neurotrophic factor (BDNF), which binds to TrkB receptors on dorsal horn neurons, altering chloride homeostasis by downregulating the potassium-chloride cotransporter (KCC2). The resultant shift in the chloride equilibrium potential reverses the inhibitory action of GABAergic and glycinergic neurotransmission, leading to disinhibition of spinal nociceptive circuits and further enhancing pain signaling. These changes at the spinal level are mirrored by altered patterns of cortical activation, as observed through functional MRI studies, where increased connectivity between the prefrontal cortex (PFC), primary motor cortex (M1), and supplementary motor area (SMA) suggests a reorganization of sensorimotor networks. This cortical plasticity, initially a compensatory mechanism to cope with the altered sensory input from the injured joint, can become maladaptive over time, resulting in aberrant motor patterns, inefficient muscle recruitment strategies, and chronic deficits in voluntary motor control.

During the later stages of recovery, typically around four to six weeks post-injury, the inflammatory environment undergoes a marked transition towards an anti-inflammatory profile, characterized by the increased expression of cytokines such as IL-10 and IL-4, which are secreted by M2 macrophages and regulatory T cells (Tregs). IL-10 signals through the IL-10 receptor (IL-10R)

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complex to activate the STAT3 pathway, leading to the upregulation of suppressor of cytokine signaling (SOCS) proteins that inhibit NF-κB and MAPK signaling, thereby reducing the expression of pro-inflammatory cytokines and chemokines. Similarly, IL-4, through its interaction with IL-4Rα and subsequent activation of STAT6, promotes the polarization of macrophages into an anti-inflammatory M2 phenotype, which secretes growth factors such as transforming growth factor-beta (TGF-β) and insulin-like growth factor-1 (IGF-1). These growth factors play a critical role in tissue repair by stimulating fibroblast proliferation, enhancing collagen synthesis, and promoting extracellular matrix remodeling. However, if this anti-inflammatory response is dysregulated, it can lead to pathological fibrosis due to excessive collagen deposition, impairing joint mobility and contributing to long-term stiffness and reduced function.

Given the dynamic interplay between these molecular mediators and the neuromuscular system, understanding the temporal dynamics of cytokine expression and their effects on nociceptive and motor pathways is critical for designing effective rehabilitation strategies. For instance, early interventions that target pro-inflammatory pathways—such as the administration of specific cytokine inhibitors (e.g., IL-1 receptor antagonist) or the use of dietary polyphenols like procyanidins—may help mitigate the acute inflammatory response, thereby preserving muscle activation and reducing pain. In contrast, during the later stages of recovery, strategies that promote the anti-inflammatory and tissue regenerative properties of IL-10 and IL-4 could enhance muscle reconditioning and restore normal motor control. Moreover, future research should prioritize the development of personalized rehabilitation protocols that take into account individual variations in cytokine profiles, genetic predispositions, and baseline neuromuscular function, thereby optimizing recovery outcomes and reducing the risk of chronic pain and functional deficits. The integration of molecular diagnostics, such as cytokine profiling and neuroimaging biomarkers, could provide a more comprehensive understanding of each patient's unique inflammatory and neuromuscular response, enabling clinicians to tailor interventions that address the specific molecular drivers of AMI and promote a more efficient and complete recovery.

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