EVs in Delivering Cytokines

Subjects: Pathology

Contributor: Lucy Norling, Mohammed Hussain

Cytokines are small proteins with well characterised effects on cell survival, migration and activation in a range of biological systems and cell types. Currently, the best described activities of cytokine are those whose delivery has been observed in a free soluble or cell tethered form. Interestingly, cytokines may also be delivered, and exert biological functions, in association with extracellular vesicles (EVs). EVs are a heterogenous population of constitutively released cellular material typically characterised by their size. EVs may for example be released by the direct blebbing of the cell membrane and in doing so, may encapsulate cellular contents. Cargo laden EVs have been found to contain a wide array of cargo, either via encapsulation or associated to the surface of EVs, and includes nucleic acids, lipid mediators and proteins, such as cytokines. EV encapsulation provides cytokine cargo protection of degradation and allows the delivery of cytokines far from the site of inflammation and injury. However, it is becoming more apparent that during pathophysiological conditions, such as those observed during autoimmunity, EVs may represent a large reservoir of ‘stealth’ cytokine that might prove difficult to target with current therapies.

Keywords: Cytokines ; Extracellular Vesicles ; Exosomes ; Microvesicles ; Autoimmunity ; IL-6 ; TNF-α ; IL-1β

1. Cytokines, Inflammation, and Autoimmunity

Secreted, soluble factors such as hormones, growth factors, and cytokines are key drivers of cellular communication. Cytokines are small, soluble proteins weighing thirty kilodaltons or less that are synthesized and secreted by a range of cells, including both immune cells, such as neutrophils, B-, and T-cells, and stromal cells, such as endothelial cells and fibroblasts [1]. The effects of cytokines are pleiotropic in nature and any given mixture or single cytokine may result in functionally different but stereotyped outcomes in a context-dependent manner [2][3]. Their soluble nature enables cytokines to act in the local microenvironment or to also exert their influence in an endocrine manner. Local cytokine activity enables cells to self-regulate their expression and secretion with many feed-forward and negative feedback loops existing in most cytokine systems/hierarchies. Cytokine release is possible in any organ and compartment throughout the body and has far reaching effects on cell survival, differentiation, and activation [4]. Interestingly, cytokine interactions with their cognate receptors are high affinity and under physiological conditions occur at picomolar concentrations and as a result, their release, and consumption, remains highly regulated [5]. Nevertheless, the half-life of any given cytokine is relatively small, and degradation in extracellular fluids or blood occurs rapidly [6]. Cytokines are also able to act in a manner independent of secretion and can act as cell surface ligands. While soluble cytokines may promote unidirectional signalling into the target cells, cell surface cytokines can initiate bi-directional outside-in signalling [7].

The key physiological function of cytokines as a group of proteins is the initiation, maintenance, and resolution of inflammatory responses [8]. Inflammation is the stereotyped, non-specific response to conserved peptides, motifs, and signals, which may be pathogens or damage-associated molecular patterns. Under non-pathological conditions, inflammatory responses are typically self-limiting and immune cell recruitment and clearance is a tightly regulated and stepwise process [9]. The initial release of pro-inflammatory cytokines, such as tumour necrosis factor alpha (TNF-α) and interleukin 1 beta (IL-1β), by tissue-resident cells induces the expression of adhesion molecules, such as selectins and integrins, which facilitates immune cell recruitment [10]. While the function of most pro-inflammatory cytokines might be functionally redundant and overlapping, certain caveats exist. For example, TNF-α promotes adhesion molecule expression on both leukocytes and endothelial cells, while IL-1β predominantly affects endothelial cells [11]. Furthermore, while TNF-α-mediated immune cell recruitment can efficiently occur independent of certain junctional proteins, recruitment through the IL-1β stimulatory pathway is decreased [12]. TNF-α is also capable of directly activating and stabilizing a pro-inflammatory phenotype in a range of cells, such as neutrophils and monocytes, which in turn stimulates them to upregulate the expression of their own range of cytokines and proteins in a self-amplifying loop [13][14]. This cycle of primary activation and secondary cytokine release can be observed with most pro-inflammatory cytokines [15][16][17].

Interleukin-6 (IL-6) levels are elevated as a result of secondary secretion in response to both TNF-α and IL-1β, interestingly, IL-6 feeds back to stimulate further TNF-α and IL-1β secretion [18]. Zheng et al., amongst others, have shown this to be true as it is possible to significantly decrease circulating IL-6, while also ameliorating clinical symptoms, in immunologically challenged mice with global deletions in IL-1β [19]. Finally, while pro-inflammatory cytokines are important for the initiation and maintenance of inflammation the appropriate anti-inflammatory signals must be integrated into any given system to terminate inflammation and promote immune cell clearance and tissue regeneration. Anti-inflammatory cytokines, such as interlekin-10 (IL-10) are able to supress pro-inflammatory gene expression and skew the phenotypic switch of immune cells away from a pro-inflammatory profile towards one that favours resolution and regeneration [3]. A number of cytokines also exert differential effects depending on the environmental context in which they are active or indeed the mechanism through which they may act. IL-6 is one such pro-inflammatory cytokine, whereby classical signalling induces anti-inflammatory effects and trans-signalling induces inflammation [3][18].

When the activity of cytokines, such as TNF-α, IL-1β, and IL-6, becomes dysregulated it is understood to be key in the pathophysiology of autoimmune diseases [20]. Autoimmunity is a broad term encompassing a range of diseases characterized by the loss of central tolerance and the maintained, pro-inflammatory immune response directed at host antigens. From an aetiological perspective, most autoimmune diseases have been reported to be multi-factorial with genetic polymorphisms infectious and environmental factors playing a role in their pathophysiology. The full spectrum of autoimmune disorders spans organ-specific to systemic diseases and includes conditions such as type 1 diabetes (T1D), rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and multiple sclerosis (MS) [21]. The major autologous antigens driving these diseases have been summarized by others (reviewed by Suurmond et al. [22]).

TNF-α is implicated across a range of autoimmune conditions and diseases and has received great attention as a therapeutic target [23]. Organ-specific increases in TNF-α have been shown to induce innate immune cell infiltration and activation, tissue resident cell activation, and pro-inflammatory cytokine secretion [24][25][26]. In particular, the blockade of TNF-α has been shown to ameliorate the inflammatory symptoms of RA and attenuate the extent of joint and cartilage erosion [27]. Similarly, increases in serum IL-6 levels have been reported as biomarkers of systemic B-cell activation, and the extent of circulating IL-6 reflected radiographic RA progression [28]. IL-6 stimulation of B-cells induces their differentiation into plasma cells and is accompanied by elevated circulating immunoglobulin levels, especially relevant in the circulating immune-complexes critical to the progression of lupus [29][30]. IL-6, as well as IL-1β, can also induce innate immune cell recruitment, and inhibit the regulatory phenotype of $CD4^+$ T-cells [31]. Levels of IL-1β have been found to be elevated in the blood, cerebrospinal fluid, and central nervous system lesions of MS patients [32]. Pro-inflammatory T-cells from patients with MS have been shown to stimulate IL-1β production from myeloid cells, which in turn drives the continued expansion of inflammatory T-cells [33]. Similar increases in systemic IL-1β have been observed in patients with RA, and the success of clinical trials for anti-IL-1β therapeutics demonstrates the significant and pathogenic role of IL-1β during autoimmunity. However, anti-IL-1β therapeutics, such as anakinra, have not managed to emulate similar success in the clinic following concerns surrounding cost and off-target effects [34]. Not only is the production of pro-inflammatory cytokines exaggerated in disease, but the activity of anti-inflammatory cytokines inhibiting them is also dysregulated. Interleukin-37 is a naturally occurring antagonist of IL-1 family member cytokines, its release by macrophages has been shown to inhibit pro-inflammatory cytokine release in mast cells, the major pathological cell type in SLE. Paradoxically, circulating IL-37 is increased in patients with SLE, and it is thought that this reflects an IL-1/IL-37 negative feedback loop in the context of mast cell insensitivity to IL-37 [35]. Nevertheless, although the role of soluble factors, such as cytokines, and physical cell–cell contact as mediators of cell communication and drivers of autoimmunity are well described, extracellular vesicles (EVs) have remained largely underappreciated, that is, until recently [36].

2. The Role of EVs in Delivering Cytokines

Immune cells can constitutively release cytokines in order to mediate homeostatic functions [37]. While it is understood that homeostatic cytokine release can occur through specific pathways of exocytosis, many have also shown that EVs are also utilized as tools for cytokine release/secretion [38]. Using a range of conditioned culture media, tissue explants, and bodily fluids, Fitzgerald et al. have shown a spectrum of heterogenous cytokine secretions in either free-soluble or EV-associated forms. Rather than EV association, or not, being a characteristic of any given cytokine, the authors have shown that, of the 33 cytokines assayed, the proportion of EV-associated versus free, soluble cytokine was dependent on the system of origin. For example, of the range of cytokines explored, the majority were found in their soluble form in placental villous explants. In contrast, the conditioned media from isolated T-cells and monocytes were found to contain the same panel of cytokines in, mostly, an EV-associated form (encapsulated or surface tethered). While on the whole, all cytokines were associated with EVs to some extent but eleven, in particular: IL-2, IL-4, IL-12p70, IL-17, IL-21, IL-22, IL-33, IFN-γ, C-X-C motif chemokine 11, transforming growth factor beta, and TNF-α, were observed to be associated with EVs more often when compared to the amount in a soluble form. The authors also found that activation altered the distribution

of cytokines between soluble and EV-associated, to the extent where different pro-inflammatory stimuli, such as lipopolysaccharide (LPS) or polyinosinic:polycytidylic acid, induced differential profiles of cytokine distribution. Interestingly, different stimuli also altered whether the range of cytokines were either EV encapsulated or surface bound. The authors have demonstrated that EV-associated cytokines maintained their functionality in reporter cell lines; however, how free, EV-encapsulated, or EV-tethered cytokines may differ functionally remains to be seen [39].

Despite demonstrating biological activity, an important question must be considered, are circulating EV-associated cytokines at levels that might be physiologically, and clinically, significant? Fitzgerald et al. have reported that levels of free IL-1β and TNF-α in plasma from healthy donors were found at 7.5 and 4.9 picogram per millilitre (pg/mL), respectively. The proportion of EV-associated IL-1β and TNF-α was observed to be comparable to the amount free, 5.5 and 6.5 pg/mL, respectively [39]. Im et al. have shown that in serum from healthy young individuals approximately 30 pg/mL of TNF-α is associated with exosomes, and the proportion found in exosomes increases 3-fold with age, one of the largest risk factors associated with autoimmunity [40]. While the specific distribution during autoimmunity is not known, in vitro experimentation can begin to shine a light on how they might be related. Cytokine-stimulated T-cells have been shown to release IL-1β and TNF-α in EV-associated forms at average concentrations of 2.7 and 1.6 pg/mL, respectively, and only 0.1 pg/mL of both cytokines was found to exist in free form [39]. The current evidence therefore suggests that cytokines exist in an EV-associated form at significant levels. However, studies comparing the level of free, soluble cytokines and EV-associated ones during autoimmunity are required. Due to EV heterogeneity, it is now understood that the levels of certain cytokines, such as monocyte chemoattractant protein-1 (MCP-1), may vary between EVs of different sizes. Using a pancreatic β-islet cell line treated with a cocktail of cytokines, Giri et al. showed that the largest EV type, apoptotic bodies, had the greatest amount of MCP-1 associated with them (358 femtogram/7.6 × 10^4 particles/1 × 10^6 cells), while medium-sized microvesicles were associated with lower amounts (127.5 femtogram/8.25 × 10^5 particles/1 × 10^6 cells) and small extracellular vesicles were associated with the least (16.4 femtogram/9.1 × 10^7 particles/1 × 10^6 cells). The authors also demonstrated a positive relationship between cytokine concentration and EV size for IFN-γ, TNF-α, and IL-1β [41]. However, further investigation is warranted on whether this data reflects a paradigm whereby greater particle size facilitates greater payload levels or a potential mechanism for selective cytokine packaging based on EV size.

Currently, one of the most well-studied cytokines in relation to its association with EVs is IL-1β. On average, across several biological systems, the abundance of IL-1β has been shown to be equally distributed between EVs and free, soluble levels [39]. Unlike most other cytokines, IL-1β lacks a signal sequence and therefore has a non-conventional secretion pathway in association with EV release [42]. Previously, IL-1β has been shown to be released associated with exosomes from dendritic cells in patients with lupus [43]. IL-1β synthesis and release via EVs is highly regulated and dependent on the activation of the NOD-like receptor family pyrin domain containing 3 inflammasome. The inflammasome is a multiprotein complex, which directs inflammatory signalling in a range of cells and its activity has been shown to be a key driver in a range of autoimmune conditions, including RA and T1D [44][45]. It has been demonstrated that non-classical secretion of IL-1β is mediated by microvesicle shedding in monocytes, macrophages, dendritic cells, and microglia and following the activation of purinergic receptors on the surface of EVs, IL-1β is released into the extracellular space [46][47] [48]. Increased purinergic receptor expression and signalling has been reported in the inflamed synovial tissue of arthritic rats and has been implicated in the pathogenesis of SLE [49][50]. Activation of synovial fibroblasts with IL-1β induces an arthritic phenotype, increasing cartilage degrading enzymes as well as IL-6 and vascular endothelial growth factor [51]. The pathogenic role of helper T-cells (Th cells) in autoimmunity has been well described; however in recent years, the role and impact of IL-17-secreting T-cells during autoimmune conditions such as RA, psoriasis, and SLE have been reported [52]. Hebel et al. have shown that IL-1β activates $CD4^+$ T-cells, in conjunction with CD3 and CD28 stimulation, causing the release of IL-17. Sustained IL-1β signalling in combination with TGF-β and/or IL-6 causes committal of T-cell differentiation into a Th-17 fate [53]. Interestingly, the authors showed that IL-1β stimulation also induced the release of IFN-γ, and others have shown that IFN-γ induces the increased shedding of EVs by increasing the activity of EV-packaging machinery, such as interferon-stimulated gene 15 [53][54]. Ultimately, IFN-γ stimulates further inflammasome activation, therefore inducing further IL-1β synthesis and release via EVs in chronic inflammation. Stimulation by IL-1 family members has been seen to induce the release of IL-6-containing EVs from mast cells in a manner independent of de-granulation [55]. While the involvement of mast cells during autoimmunity is debated by some, data exists to support their pathogenic role in RA and multiple sclerosis [56]. In recent years, an autoimmune component has been implicated in the pathogenesis of amyotrophic lateral sclerosis and the release of EVs containing IL-6 from astrocytes is thought to contribute to disease pathogenesis and activity [57][58].

Systemic levels of IL-6, in part regulated by the packaging and secretion of microvesicles, have been shown to be increased in a range of autoimmune conditions [59]. Interestingly, multiple mechanisms of IL-6 signalling exist, these include traditional ligand–receptor interactions, receptor trans-signalling, and finally, IL-6/IL-6 receptor trans-presentation [60]. Moreover, IL-6 receptor expression has been reported on the surface of EVs [61]. Arnold et al. demonstrated that EV-

bound IL-6 receptors can be donated to cells lacking receptor expression by vesicular fusion. This process of IL-6 receptor transmission has been termed joint reconstituted signalling and, inherently, increases the bioactivity of circulating IL-6 on a greater range of cells [62]. Evidence exists for the relevance of IL-6 signalling in inducing T-cell homing to pancreatic islets during T1D. However, this was not related to any increases in T-cell IL-6 levels, as observed by mRNA levels, but the increased expression of IL-6 receptor on the surface of T-cells [63]. Indeed, increased IL-6 signalling in T-cells has been shown to contribute to T-cell differentiation into IL-17-secreting cells and resistance to regulatory T-cell differentiation and their effector functions [64]. More recently, a role for the gut microbiota and changes in systemic LPS levels were found to drive the pathogenesis of autoimmune conditions [65]. To this end, Obregon et al. have shown that LPS-stimulated dendritic cells undergo exosome release, and these exosomes contain TNF-α, as well as, MHC-II, CD40, and CD83 [66]. Others have shown that exosomal-tethered TNF-α derived from mature dendritic cells induces endothelial inflammation [67]. Interestingly, Zhang et al. have shown that exosomes with a surface-tethered form of TNF-α, isolated from the synovial fibroblasts of RA patients, were also able to stimulate T-cells and in doing so, made them resistant to activation-induced cell death [68]. Liu et al. have reported similar effects of TNF-α associated exosomes on T-cells in Crohn's disease [69]. The current evidence suggests that EVs make robust vessels for the delivery of cytokines, at levels that are clinically releavant during autoimmity. Therefore, is it possible to modulate EV activity in order to develop a therapeutic tool for treating autoimmunity?

References

1. Deverman, B.E.; Patterson, P.H. Cytokines and CNS Development. Neuron 2009, 64, 61–78, doi:10.1016/j.neuron.2009.09.002.
2. Lacy, P.; Stow, J.L. Cytokine release from innate immune cells: Association with diverse membrane trafficking pathways. Blood 2011, 118, 9–18, doi:10.1182/blood-2010-08-265892.
3. Dinarello, C.A. Historical insights into cytokines. Eur. J. Immunol. 2007, 37, S34–S45, doi:10.1002/eji.200737772.
4. Yoshida, T.; Satoh, M.; Nakagaito, Y.; Kuno, H.; Takeuchi, M. Cytokines affecting survival and differentiation of an astrocyte progenitor cell line. Dev. Brain Res. 1993, 76, 147–150, doi:10.1016/0165-3806(93)90132-t.
5. Wilson, C.J.; Finch, C.E.; Cohen, H.J. Cytokines and cognition—The case for a head-to-toe inflammatory paradigm. J. Am. Geriatr. Soc. 2002, 50, 2041–2056, doi:10.1046/j.1532-5415.2002.50619.x.
6. Aziza, N.; Detels, R.; Quint, J.J.; Li, Q.; Gjertson, D.; Butch, A.W. Stability of cytokines, chemokines and soluble activation markers in unprocessed blood stored under different conditions. Cytokine 2016, 84, 17–24, doi:10.1016/j.cyto.2016.05.010.
7. Liongue, C.; Sertori, R.; Ward, A.C. Evolution of Cytokine Receptor Signaling. J. Immunol. 2016, 197, 11–18, doi:10.4049/jimmunol.1600372.
8. Hopkins, S.J. The pathophysiological role of cytokines. Leg. Med. 2003, 5, S45–S57, doi:10.1016/s1344-6223(02)00088-3.
9. Ley, K.; Laudanna, C.; Cybulsky, M.I.; Nourshargh, S. Getting to the site of inflammation: The leukocyte adhesion cascade updated. Nat. Rev. Immunol. 2007, 7, 678–689, doi:10.1038/nri2156.
10. Dunne, J.L.; Ballantyne, C.M.; Beaudet, A.L.; Ley, K. Control of leukocyte rolling velocity in TNF-α–induced inflammation by LFA-1 and Mac-1. Blood 2002, 99, 336–341, doi:10.1182/blood.v99.1.336.
11. Rampart, M.; Fiers, W.; Smet, W.; Herman, A.G. Different pro-inflammatory profiles of interleukin 1 (IL 1) and tumor necrosis factor (TNF) in anin vivo model of inflammation. Inflamm. Res. 1989, 26, 186–188, doi:10.1007/bf02126603.
12. Nourshargh, S.; Krombach, F.; Dejana, E. The role of JAM-A and PECAM-1 in modulating leukocyte infiltration in inflamed and ischemic tissues. J. Leukoc. Biol. 2006, 80, 714–718, doi:10.1189/jlb.1105645.
13. Gane, J.M.; Stockley, R.A.; Sapey, E. TNF-αAutocrine Feedback Loops in Human Monocytes: The Pro- and Anti-Inflammatory Roles of the TNF-αReceptors Support the Concept of Selective TNFR1 BlockadeIn Vivo. J. Immunol. Res. 2016, 2016, 1–13, doi:10.1155/2016/1079851.
14. Deguine, J.; Wei, J.; Barbalat, R.; Gronert, K.; Barton, G.M. Local TNFR1 Signaling Licenses Murine Neutrophils for Increased TLR-Dependent Cytokine and Eicosanoid Production. J. Immunol. 2017, 198, 2865–2875, doi:10.4049/jimmunol.1601465.
15. Eskan, M.A.; Benakanakere, M.R.; Rose, B.G.; Zhang, P.; Zhao, J.; Stathopoulou, P.; Fujioka, D.; Kinane, D.F. Interleukin-1β Modulates Proinflammatory Cytokine Production in Human Epithelial Cells. Infect. Immun. 2008, 76, 2080–2089, doi:10.1128/iai.01428-07.

16. Madej, M.P.; Töpfer, E.; Boraschi, D.; Italiani, P. Different Regulation of Interleukin-1 Production and Activity in Monocyt es and Macrophages: Innate Memory as an Endogenous Mechanism of IL-1 Inhibition. Front. Pharmacol. 2017, 8, 335, doi:10.3389/fphar.2017.00335.

17. Ben-Sasson, S.Z.; Hu-Li, J.; Quiel, J.; Cauchetaux, S.; Ratner, M.; Shapira, I.; Dinarello, C.A.; Paul, W.E. IL-1 acts dire ctly on CD4 T cells to enhance their antigen-driven expansion and differentiation. Proc. Natl. Acad. Sci. USA 2009, 106, 7119–7124.

18. Narazaki, M.; Kishimoto, T. The Two-Faced Cytokine IL-6 in Host Defense and Diseases. Int. J. Mol. Sci. 2018, 19, 352 8, doi:10.3390/ijms19113528.

19. Zheng, H.; Fletcher, D.; Kozak, W.; Jiang, M.; Hofmann, K.J.; Corn, C.A.; Soszynski, D.; Grabiec, C.; Trumbauer, M.E.; Shaw, A.; et al. Resistance to fever induction and impaired acute-phase response in interleukin-1β-deficient mice. Imm unity 1995, 3, 9–19, doi:10.1016/1074-7613(95)90154-x.

20. Lamkanfi, M.; Dixit, V.M. Inflammasomes and Their Roles in Health and Disease. Annu. Rev. Cell Dev. Biol. 2012, 28, 1 37–161, doi:10.1146/annurev-cellbio-101011-155745.

21. Rosenblum, M.D.; Remedios, K.A.; Abbas, A.K. Mechanisms of human autoimmunity. J. Clin. Investig. 2015, 125, 2228 –2233, doi:10.1172/jci78088.

22. Suurmond, J.; Diamond, B. Autoantibodies in systemic autoimmune diseases: Specificity and pathogenicity. J. Clin. Inv estig. 2015, 125, 2194–2202, doi:10.1172/jci78084.

23. Fragoso, J.M.; Vargas-Alarcón, G.; Jiménez-Morales, S.; Hernández, O.D.R.; Bello, J.R. Tumor necrosis factor alpha (TNF-?) in autoimmune diseases (AIDs): Molecular biology and genetics. Gac. Med. Mex. 2014, 150, 334–344.

24. Kassiotis, G.; Kollias, G. TNF and receptors in organ-specific autoimmune disease: Multi-Layered functioning mirrored i n animal models. J. Clin. Investig. 2001, 107, 1507–1508, doi:10.1172/JCI13362.

25. Wang, K.; Ye, L.; Lu, H.; Chen, H.; Zhang, Y.; Huang, Y.; Zheng, J. TNF-α promotes extracellular vesicle release in mou se astrocytes through glutaminase. J. Neuroinflamm. 2017, 14, 87, doi:10.1186/s12974-017-0853-2.

26. Aringer, M.; Smolen, J.S. The role of tumor necrosis factor-alpha in systemic lupus erythematosus. Arthritis Res. Ther. 2008, 10, 202, doi:10.1186/ar2341.

27. Toussirot, E.; Wendling, D. The use of TNF-α blocking agents in rheumatoid arthritis: An overview. Expert Opin. Pharm acother. 2004, 5, 581–594, doi:10.1517/14656566.5.3.581.

28. Gottenberg, J.E.; Dayer, J.M.; Lukas, C.; Ducot, B.; Chiocchia, G.; Cantagrel, A.; Saraux, A.; Roux-Lombard, P.; Mariett e, X. Serum IL-6 and IL-21 are associated with markers of B cell activation and structural progression in early rheumato id arthritis: Results from the ESPOIR cohort. Ann. Rheum. Dis. 2012, 71, 1243–1248, doi:10.1136/annrheumdis-2011-2 00975.

29. Lipsky, P.E. The control of antibody production by immunomodulatory molecules. Arthritis Rheum. 1989, 32, 1345–135 5, doi:10.1002/anr.1780321102.

30. Phan, T.G.; Toong, C.; Adelstein, S. Clearing the complexity: Immune complexes and their treatment in lupus nephritis. Int. J. Nephrol. Renov. Dis. 2011, 4, 17–28, doi:10.2147/IJNRD.S10233.

31. Franco, P.; Laura, F.; Valentina, C.; Simona, A.; Gloria, A.; Eleonora, N.; Pandolfi, F.; Franza, L.; Carusi, V.; Altamura, S.; et al. Interleukin-6 in Rheumatoid Arthritis. Int. J. Mol. Sci. 2020, 21, 5238, doi:10.3390/ijms21155238.

32. Lin, C.-C.; Edelson, B.T. New Insights into the Role of IL-1β in Experimental Autoimmune Encephalomyelitis and Multipl e Sclerosis. J. Immunol. 2017, 198, 4553–4560, doi:10.4049/jimmunol.1700263.

33. Jain, A.; Irizarry-Caro, R.A.; McDaniel, M.M.; Chawla, A.S.; Carroll, K.R.; Overcast, G.R.; Philip, N.H.; Oberst, A.; Cherv onsky, A.V.; Katz, J.D.; et al. T cells instruct myeloid cells to produce inflammasome-independent IL-1β and cause autoi mmunity. Nat. Immunol. 2019, 21, 65–74, doi:10.1038/s41590-019-0559-y.

34. E Furst, D. Anakinra: Review of recombinant human interleukin-I receptor antagonist in the treatment of rheumatoid art hritis. Clin. Ther. 2004, 26, 1960–1975, doi:10.1016/j.clinthera.2004.12.019.

35. Caraffa, A.; Gallenga, C.E.; Kritas, S.K.; Ronconi, G.; Conti, P. Impact of mast cells in systemic lupus erythematosus: c an inflammation be inhibited? J. Bio.l Regul. Homeost Agents 2019, 33, 669–673.

36. Raposo, G.; Stahl, P.D. Extracellular vesicles: A new communication paradigm? Nat. Rev. Mol. Cell Biol. 2019, 20, 509 –510, doi:10.1038/s41580-019-0158-7.

37. Stow, J.L.; Murray, R.Z. Intracellular trafficking and secretion of inflammatory cytokines. Cytokine Growth Factor Rev. 2 013, 24, 227–239, doi:10.1016/j.cytogfr.2013.04.001.

38. Aiello, A.; Giannessi, F.; Percario, Z.A.; Affabris, E. An emerging interplay between extracellular vesicles and cytokines. Cytokine Growth Factor Rev. 2020, 51, 49–60, doi:10.1016/j.cytogfr.2019.12.003.

39. Fitzgerald, W.; Freeman, M.L.; Lederman, M.M.; Vasilieva, E.; Romero, R.; Margolis, L. A System of Cytokines Encaps ulated in ExtraCellular Vesicles. Sci. Rep. 2018, 8, 8973, doi:10.1038/s41598-018-27190-x.

40. Im, K.; Baek, J.; Kwon, W.S.; Rha, S.Y.; Hwang, K.W.; Kim, U.; Min, H. The Comparison of Exosome and Exosomal Cyt okines between Young and Old Individuals with or without Gastric Cancer. Int. J. Gerontol. 2018, 12, 233–238, doi:10.1 016/j.ijge.2018.03.013.

41. Giri, K.R.; De Beaurepaire, L.; Jegou, D.; Lavy, M.; Mosser, M.; Dupont, A.; Fleurisson, R.; Dubreil, L.; Collot, M.; Van E ndert, P.; et al. Molecular and functional diversity of distinct subpopulations of extracellular vesicles from stressed pancr eatic beta cells: Implications for autoimmunity. BioRxiv 2020, doi:10.1101/2020.03.26.003145.

42. Martín-Sánchez, F.; Diamond, C.; Zeitler, M.; Gomez, A.I.; Baroja-Mazo, A.; Bagnall, J.; Spiller, D.; White, M.; Daniels, M.; Mortellaro, A.; et al. Inflammasome-dependent IL-1β release depends upon membrane permeabilisation. Cell Deat h Differ. 2016, 23, 1219–1231, doi:10.1038/cdd.2015.176.

43. Pérez-Hernández, J.; Cortes, R. Extracellular Vesicles as Biomarkers of Systemic Lupus Erythematosus. Dis. Markers 2015, 2015, 613536, doi:10.1155/2015/613536.

44. Ding, S.; Xu, S.; Ma, Y.; Liu, G.; Jang, H.; Fang, J. Modulatory Mechanisms of the NLRP3 Inflammasomes in Diabetes. Biomolecules 2019, 9, 850, doi:10.3390/biom9120850.

45. Yi, Y.-S. Role of inflammasomes in inflammatory autoimmune rheumatic diseases. Korean J. Physiol. Pharmacol. 2017, 22, 1–15, doi:10.4196/kjpp.2018.22.1.1.

46. MacKenzie, A.; Wilson, H.L.; Kiss-Toth, E.; Dower, S.K.; North, R.; Surprenant, A. Rapid Secretion of Interleukin-1β by Microvesicle Shedding. Immunity 2001, 15, 825–835, doi:10.1016/s1074-7613(01)00229-1.

47. Qu, Y.; Franchi, L.; Nunez, G.; Dubyak, G.R. Nonclassical IL-1β Secretion Stimulated by P2X7 Receptors Is Dependent on Inflammasome Activation and Correlated with Exosome Release in Murine Macrophages. J. Immunol. 2007, 179, 19 13–1925, doi:10.4049/jimmunol.179.3.1913.

48. Pizzirani, C.; Ferrari, D.; Chiozzi, P.; Adinolfi, E.; Sandonà, D.; Savaglio, E.; Di Virgilio, F. Stimulation of P2 receptors ca uses release of IL-1β–loaded microvesicles from human dendritic cells. Blood 2006, 109, 3856–3864, doi:10.1182/bloo d-2005-06-031377.

49. McInnes, I.B.; Cruwys, S.; Bowers, K.; Braddock, M. Targeting the P2X7 receptor in rheumatoid arthritis: Biological ratio nale for P2X7 antagonism. Clin. Exp. Rheumatol. 2014, 32, 878–882.

50. Di Virgilio, F.; Giuliani, A.L. Purinergic signalling in autoimmunity: A role for the P2X7R in systemic lupus erythematosu s? Biomed. J. 2016, 39, 326–338, doi:10.1016/j.bj.2016.08.006.

51. Kato, T.; Miyaki, S.; Ishitobi, H.; Nakamura, Y.; Nakasa, T.; Lotz, M.K.; Ochi, M. Exosomes from IL-1β stimulated synovi al fibroblasts induce osteoarthritic changes in articular chondrocytes. Arthritis Res. 2014, 16, R163, doi:10.1186/ar467 9.

52. Waite, J.C.; Skokos, D. Th17 Response and Inflammatory Autoimmune Diseases. Int. J. Inflamm. 2011, 2012, 819467, doi:10.1155/2012/819467.

53. Hebel, K.; Rudolph, M.; Kosak, B.; Chang, H.-D.; Butzmann, J.; Brunner-Weinzierl, M.C. IL-1β and TGF-β Act Antagoni stically in Induction and Differentially in Propagation of Human Proinflammatory Precursor CD4+ T Cells. J. Immunol. 2 011, 187, 5627–5635, doi:10.4049/jimmunol.1003998.

54. Hotter, D.; Kirchhoff, F. Interferons and beyond: Induction of antiretroviral restriction factors. J. Leukoc. Biol. 2017, 103, 465–477, doi:10.1002/jlb.3mr0717-307r.

55. Kandere-Grzybowska, K.; Letourneau, R.; Kempuraj, D.; Donelan, J.; Poplawski, S.; Boucher, W.; Athanassiou, A.; The oharides, T.C. IL-1 induces vesicular secretion of IL-6 without degranulation from human mast cells. J. Immunol. 2003, 171, 4830–4836, doi:10.4049/jimmunol.171.9.4830.

56. Brown, M.A.; Hatfield, J.K. Mast Cells are Important Modifiers of Autoimmune Disease: With so Much Evidence, Why is There Still Controversy? Front. Immunol. 2012, 3, 147, doi:10.3389/fimmu.2012.00147.

57. Pagani, M.R.; Gonzalez, L.E.; Uchitel, O.D. Autoimmunity in Amyotrophic Lateral Sclerosis: Past and Present. Neurol. Res. Int. 2011, 2011, 497080, doi:10.1155/2011/497080.

58. Chen, Y.; Xia, K.; Chen, L.; Fan, D. Increased Interleukin-6 Levels in the Astrocyte-Derived Exosomes of Sporadic Amy otrophic Lateral Sclerosis Patients. Front. Mol. Neurosci. 2019, 13, 13, doi:10.3389/fnins.2019.00574.

59. Barnes, B.J.; Somerville, C.C. Modulating Cytokine Production via Select Packaging and Secretion from Extracellular V esicles. Front. Immunol. 2020, 11, 11, doi:10.3389/fimmu.2020.01040.

60. Garbers, C.; Heink, S.; Korn, T.; Rose-John, S. Interleukin-6: Designing specific therapeutics for a complex cytokine. N at. Rev. Drug Discov. 2018, 17, 395–412, doi:10.1038/nrd.2018.45.

61. Schumacher, N.; Meyer, D.; Mauermann, A.; Von Der Heyde, J.; Wolf, J.; Schwarz, J.; Knittler, K.; Murphy, G.; Michalek, M.; Garbers, C.; et al. Shedding of Endogenous Interleukin-6 Receptor (IL-6R) Is Governed by A Disintegrin and Metalloproteinase (ADAM) Proteases while a Full-length IL-6R Isoform Localizes to Circulating Microvesicles. J. Biol. Chem. 2015, 290, 26059–26071, doi:10.1074/jbc.M115.649509.
62. Arnold, P.; Lückstädt, W.; Li, W.; Boll, I.; Lokau, J.; Garbers, C.; Lucius, R.; Rose-John, S.; Becker-Pauly, C. Joint Reconstituted Signaling of the IL-6 Receptor via Extracellular Vesicles. Cells 2020, 9, 1307, doi:10.3390/cells9051307.
63. Bettelli, E.; Carrier, Y.; Gao, W.; Korn, T.; Strom, T.B.; Oukka, M.; Weiner, H.L.; Kuchroo, V.K. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature 2006, 441, 235–238, doi:10.1038/nature04753.
64. Gomes, K.B. IL-6 and type 1 diabetes mellitus: T cell responses and increase in IL-6 receptor surface expression. Ann. Transl. Med. 2017, 5, 16, doi:10.21037/atm.2016.12.74.
65. Feehley, T.; Belda-Ferre, P.; Nagler, C.R. What's LPS Got to Do with It? A Role for Gut LPS Variants in Driving Autoimmune and Allergic Disease. Cell Host Microbe 2016, 19, 572–574, doi:10.1016/j.chom.2016.04.025.
66. Obregon, C.; Rothen-Rutishauser, B.; Gerber, P.; Gehr, P.; Nicod, L.P. Active Uptake of Dendritic Cell-Derived Exovesicles by Epithelial Cells Induces the Release of Inflammatory Mediators through a TNF-α-Mediated Pathway. Am. J. Pathol. 2009, 175, 696–705, doi:10.2353/ajpath.2009.080716.
67. Gao, W.; Liu, H.; Yuan, J.; Wu, C.; Huang, N.; Ma, Y.; Zhu, J.; Ma, L.; Guo, J.; Shi, H.; et al. Exosomes derived from mature dendritic cells increase endothelial inflammation and atherosclerosis via membrane TNF-α mediated NF-κB pathway. J. Cell. Mol. Med. 2016, 20, 2318–2327, doi:10.1111/jcmm.12923.
68. Zhang, H.-G.; Liu, C.; Su, K.; Yu, S.; Zhang, L.; Zhang, S.; Wang, J.; Cao, X.; Grizzle, W.; Kimberly, R.P. A Membrane Form of TNF-α Presented by Exosomes Delays T Cell Activation-Induced Cell Death. J. Immunol. 2006, 176, 7385–7393, doi:10.4049/jimmunol.176.12.7385.
69. Liu, H.; Liang, Z.; Wang, F.; Zheng, X.; Zeng, Z.; He, X.; Gao, X.; Zhi, M.; Wu, X.; Wu, X.; et al. Intestinal CD14+ Macrophages Protect CD4+ T Cells From Activation-induced Cell Death via Exosomal Membrane TNF in Crohn's Disease. J. Crohns Colitis 2020, doi:10.1093/ecco-jcc/jjaa083.
