Why we should treat COVID-19 Long-Hauler Syndromes with Convalescent Plasma; Contained Suppressive Exosomes are likely COVID antigen-specific

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Abstract

There have been numerous very disappointing results of Convalescent Plasma Therapy (CPT) in active infections with COVID-19 virus, raises a question of how to account for this, given the huge history of seeming benefit of CPT in a variety of infectious diseases over more than the past 100 years. We propose the following as a possible explanation, based on our experimental evidence. In CPT there is a collision between developed desirable viral resistance promoting hyper-immune antibodies and undesirable convalescent exosomes antigen (Ag)-specifically suppressing cellular immune responses stimulated by the prior now recovered acute viral disease. These inhibiting exosomes, that act to suppress Ag presenting cells and anti-COVID-19-Ag-specific effector T cells, are appropriate to convalescence, but when given early in infection may interfere with endogenous early developing profitable cellular immune anti-viral responses. To account for the high incidence of the Long Haulers post COVID patients, we postulate that these are due to immune reactivity to Ag remnants of the virus and not residual infection. These are postulated to held by and augmented by remnants of highly pathogenetic neutrophil extracellular traps (NETs). We propose that CPT with its content of potential broadly COVID Ag-specific suppressive exosomes be considered for possible effective treatment of the COVID-19 Long Hauler Syndromes. This certainly is so compared to the purported value of therapy with vaccines, as the diverse Ag-specific extracellular vesicles in the convalescent plasma would be an inhibitory influence on multiple COVID Ag-specific responses, beyond just to the spike protein of the virus.

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"Why we should treat COVID-19 Long Hauler Syndromes with Convalescent Plasma; Contained Suppressive Exosomes are likely COVID antigen-specific"

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Short title:

COVID-19 convalescent plasma therapy contains COVID antigen-specific exosomes for inhibiting persisting immune responses to residual Ag in patients with LHS

Keywords:

COVID-19, exosomes, extracellular vesicles, extracellular RNAs, convalescent plasma, miRNA, COVID antigen specific exosomes, neutrophil extracellular traps, chronic fatigue syndrome, delayed-type hypersensitivity, long hauler syndromes,

Summary sentence:

Convalescent plasma therapy from patients completely surviving COVID-19 acute viral infections likely contain immunosuppressive COVID antigen-specific exosomes able to inhibit persisting immune responses to residual antigen of COVID-19 virus in patients with LHS

ABBREVIATIONS

Ab, antibody

Ag, antigen

Ag/MHC, complex of Ag-peptide and MHC on the surface of APC

APC, antigen presenting cell

ARDS, acute respiratory distress syndrome

B cell, antibody producing lymphocyte

CFS, chronic fatigue syndrome

CNS, central nervous system

COVID-19, Coronavirus Sars-CoV-2

CTL, $CD8^{pos}$ cytotoxic T lymphocyte

DC, dendritic cell

DTH, delayed-type hypersensitivity

EBV, Epstein Barr Virus

EV, extracellular vesicle

FLC, free Ab light chain

IFN-γ, interferon gamma

IL, Interleukin

IP, intraperitoneal

IV, intravenous

LHS, long hauler syndrome

MCP, macrophage chemotactic protein

ME, Myalgic Encephalomyelitis

miRNA, micro (small) RNA

MOG, glycoprotein uniquely expressed in oligodendrocytes of the CNS

mRNA, messenger RNA

NETs, neutrophil extracellular trap

PMN, polymorphonuclear cells

ROS, reactive oxygen species

SARS, severe acute respiratory syndrome

SLE, systemic lupus erythematosis

T cell, thymic-derived lymphocyte

TCR, T cell receptor for antigen

TLR, toll-like receptors

TGF-β, transforming growth factor-beta

Th1, T helper-1 cells

Th17, T helper-17 cells

TLR, toll like receptors

TNF-α, tumor necrosis factor-alpha

ABSTRACT

There have been numerous very disappointing results of Convalescent Plasma Therapy (CPT) in active infections with COVID-19 virus, raises a question of how to account for this, given the huge history of *seeming* benefit of CPT in a variety of infectious diseases over more than the past 100 years. We propose the following as a possible explanation, based on our experimental evidence. In CPT there is a collision between developed desirable viral resistance promoting hyper-immune antibodies and undesirable convalescent exosomes antigen (Ag)-specifically suppressing cellular immune responses stimulated by the prior now recovered acute viral disease. These inhibiting exosomes, that act to suppress Ag presenting cells and anti-COVID-19-Ag-specific effector T cells, are appropriate to convalescence, but when given early in infection may interfere with endogenous early developing profitable cellular immune anti-viral responses.

To account for the high incidence of the Long Haulers post COVID patients, we postulate that these are due to immune reactivity to Ag remnants of the virus and not residual infection. These are postulated to held by and augmented by remnants of highly pathogenetic neutrophil extracellular traps (NETs). We propose that CPT with its content of potential broadly COVID Ag-specific suppressive exosomes be considered for possible effective treatment of the COVID-19 Long Hauler Syndromes. This certainly is so compared to the purported value of therapy with vaccines, as the diverse Ag-specific extracellular vesicles in the convalescent plasma would be an inhibitory influence on multiple COVID Ag-specific responses, beyond just to the spike protein of the virus.

Introduction

Clinical resistance to the benefits of COVID-19 convalescent plasma therapy derived from patients recovered from the acute active viral infections.

There is surprising overall ineffectiveness of convalescent plasma therapy (CPT) for COVID-19 infections, given much apparent success in a variety of prior serious systemic viral infections, that stretch over the past hundred years in thousands of patients **[1]**. More to the point here is the reported benefit of CPT in COVID-19 patients of several non-randomized case studies **[2,3,4,5].** Effectiveness of CPT in clinical hospitalized COVID-19 viral infections has been found to apply only to mildly sick patients (without airway intubation and on ventilators), those of certain ages **[6],** and those treated only very early in the course of disease **[7].**

Despite high enthusiasm of early analyses summarizing past pre-COVID uses **[8],** and uncontrolled COVID-19 studies **[9,10,11]**, along with several other encouraging but uncontrolled case series **[12,13,14]**, there has followed an increasing number of well controlled randomized studies from throughout the world, that clearly have demonstrated an overall ineffectiveness of CPT **[15,16,17,18,19]**. Consequently some prominent ongoing trials have been halted in The Netherlands [**16],** the extensive UK RECOVERY trial and USA NIH trials in Emergency Departments, Out patients and in those with mild to moderate disease.

The simple explanation given is that there is insufficient Ab to meet the needs of the patients with severe illness after an early susceptibility window, when the pathology then piles on and thus becomes no longer susceptible to the benefits of CPT. However, attempts to cover that focus on CPT by using donated plasma with the highest titers, that have been determined by in vitro anti-infective antibody (Ab) assays against the growth in culture of the COVID-19 virus, similarly have provided weak overall protective results **[20,21].**

For an update, note a recent randomized, double-blind, controlled trial of convalescent vs. normal control plasma in adults with severe COVID-19 showing no clinical benefit, but curiously a definite benefit per mortality [**22].** Another recent study showed remarkable improvement in cytokine storm and disease severity, but CPT did not considerably affect the mortality rate [**19].**

Conclusion: Regarding expectations of CPT in COVID-19 acute viral syndromes, we are now disappointed to instead call the overall results as an apparent clinical resistance to the purported benefits of CPT.

Consequently, the major question that emerges from these experiences is: given the vast literature and prior experiences demonstrating the efficacy of CPT in a variety of infectious illnesses, why are current success in severely ill COVID-19 patients so limited to certain patient subsets and just the short early duration of illness? *Very pointedly, we raise a particular question: is there a mechanism of immune resistance to CPT mediated by the billions of convalescent exosomes per milliliter that accompany the potentially potent helpful hyperimmune Ab in the convalescent plasma that is used to treat the actively infected patients.*

OUR HYPOTHESIS: Our analysis that follows is that in CPT there is a collision between contained desirable viral resistance promoting developed hyper-immune antibodies and undesirable convalescent cellular immune suppressing exosomes. Importantly, these inhibiting exosomes are appropriate to convalescence, but when given in the CPT early in infection would interfere with endogenous early developing profitable T cell mediated anti-viral responses. *Therefore, we postulate that convalescent plasma contains a deleterious mixture of helpful hyperimmune Ab along with also late phase inhibitory exosomes that are antagonistic, particularly when given early in infection, since they interfere with the early establishment of effective cell mediated immunity against the virus.*

Terminology for various extracellular vesicle subsets like exosomes

There is a large subgroup among the multiple extracellular vesicle (EV) subsets called exosomes that are responsible for the vast majority of transferred effects of EV that mainly transfer miRNAs **[1].** Exosomes are nano sized vesicles pinched off from intracellular membranes of the terminal endosomes near the cell surface that are 50-150 nanometers in diameter and after release by the cells pellet when ultracentrifuged at 100,000g. Their cargo can contain many subtypes of RNAs to mediate biologic effects in the targeted cells near and far away that accept exosome transfers.
In contrast, another major subset of EV are micro vesicles (MV) that originate by pinching off singly from the donor cell surface membrane, resulting in release of individual vesicles. These large MV are 200-1500 nanometers in diameter, pellet with centrifugation at only 10,000g, and thus far mediate a minority of EV transferred effects**.**

There is increased appreciation of COVID-19 “long haulers” with significant illnesses emerging after active viral infection.

This subset of patients seemingly recovered from COVID-19 serious viral illnesses tend to have lingering problems, but are not exclusively a continuation of significant signs and symptoms after successful protective immune responses have eliminated all traces of laboratory determined and symptomatic active viral infection. Those individuals are often referred to as “COVID long-haulers” and have a condition called Long COVID-19 Syndrome or “Long COVID. ”Here, we will call these Long Hauler Syndromes, not Post Covid Syndromes as many emerge as a continuation of clinical problems prominent during active infectious disease rather than appearing with an appreciable gap in between that “post-COVID” implies.

We propose here that the convalescent plasma effects are COVID-19 Ag-dependent with prominent involvement of antigen-specific exosomes

There are two potentially involved varieties of immunologic antigen (Ag)-specific exosomes. The first are mini-antigen presenting cell (APC)-like exosomes with surface expression of the involved COVID-19-peptide-Ag/MHC complex specific for the ab-T cell receptor (TCR) of the anti-COVID-19 T cells mediating the anti-COVID-19 immune response. This variety of Ag-specific exosomes can be secreted by professional APC **[23],** like dendritic cells (DC) **[24,25,26,27]** or B cells **[28],** or derived from monocyte/macrophages **[29,30,31]**, and even can be produced by epithelial cells **[32,33].** Importantly the DC and B cell-derived Ag/MHC-specific exosomes can activate companion T cells via interaction with their ab-TCR at the immune synapse by transferring their contained extracellular RNAs (exRNAs); thus acting as mini-APC. Indeed, there are numerous instances of such genetic information transfers in these interactions. The Ag/MHC-specific exosomes mostly transfer exRNAs like miRNAs to the recipient T cells, acting to mediate crucial epigenetic changes, resulting in significant functional alterations **[34,35].**

The second example of Ag-specific exosomes is when the Ag-specificity is due to surface bound specific Ab on these EV that of course also have the ability to transfer function altering miRNAs. Unusually in this most important second instance, the exosome surface Ab is derived free Ab light chains (FLC); interestingly without Ab heavy chains nor whole immunoglobulin molecules. These B cell-derived Ab FLC-expressing exosomes may be expressed on exosomes derived from T cells, in the absence of Fc receptors (note Ig light chains have no Fc portion**) [36**,**37]**, or the surface of exosomes derived from B cells [**38]**, and further can mast cells to mediate functions; also independent of Fc receptors [**39,40,41],** giving potential bioactivity analogous to IgE Ab **[40,41,42,43,44,45].**

The Ab FLC expressing Ag-specific exosomes often deliver genetic information like via miRNAs to macrophage **APC [31,42**,**45,46,47]** by binding peptide of Ag in surface compez wit **MHC [38].** Besides the ability of the Ag-specific Ab coated suppressive exosomes to inhibit APC function for subsequent activation of effector T cells, they also impair macrophage ability to induce Ab immune responses **30,** and enhance macrophage generation of reactive oxygen intermediates [**48].**

Conclusion: Convalescent plasma contains two different Ag-specific exosomes that in patients with LHS can drive COVID-19 Ag-dependent non-infectious long COVID LHS.

The first noted peptide Ag/MHC-expressing Ag-specific exosomes can act as unique immunogenic inducers of immune responses

An important postulated attribute of COVID-19 induced LHS discussed here is Ag-driven continuation of immune responses post infection. Regarding this, it has been determined that exosomes derived from DC can substitute for the whole DC to mediate immunization of Ag-specific T cells. Further, non-specific $CD4^{pos}$ T cells with uptake of Ag/MHC-specific DC-released exosomes can then stimulate antigen/MHC-specific $CD8^{pos}$ cytotoxic T cell responses and long-term Th cell memory **[25].** Thus, since the the first mentioned Ag-specific exosomes expressing peptide/MHC surface complexes are derived from APC they can act as mini-APC. If the crucial immunogenetic peptide is known, plain exosomes derived from DC derived can be pulsed with that peptide and then used as a powerful quite specific peptide/MHC surface Ag-specific immunizing agent even without use of adjuvants **[49,50].**

Alternatively, and quite importantly, if the crucial specific immunogenetic peptides are not known, say for complex immunogens like a cancer cell or an infectious agent like COVID-19, the DCs can be exposed to the whole cancer cell or infectious agent and then with subsequent incubation these DC

will produce exosomes expressing the crucial immunogenic peptides complexed with the correct MHC to then be used similarly as a powerful quite Ag-specific mini-APC immunogens; acting in part by transferring exRNAs [**51].** This pathway is employed in immunization for a variety of complex infectious agents [**52**], parasitic worms [**53,54],** and for cancer cells as well [**49,55].**

Importantly, and quite relevant here, is the use of exosomes loaded with viral Ag **[56,57],** especially SARS-CoV-2 Spike protein **[58,59,60],** as potent anti-viral immunogens . A major prediction of this review is the following. After protective immune clearing of COVID-19 virus mediating active COVID-19 infections there are remaining immunogenic Ag/MHC-specific exosomes, acting as mini-APC, that can account for the carryover of immunogenic COVID Ag. Therefore, this enables continued stimulation of non-protective persisting clinically evident immune responses to COVID Ag in LHS patients. These persisting Ag-loaded, Ag-specific stimulatory exosome immunogens then drive LHS as they may be be complexed in the remains of formed Neutrophil Extracellular Traps (NETs) [**61,62],** that are left from the pathogenesis of the acute COVID-active viral disease to S.

Alternatively, these Ag-specific Ag-immunizing Ag-specific exosomes may be present intracellularly in targeted cells like APC, persisting in phagolysosomes because of the ability of some particularly activated exosomes, like those in milk that can pass the stomach for subsequent systemic absorbtion after oral administration **[63,64,65,66,67,68],** or similarly immunosuppressive Ab FLC coated exosomes that in the orally treated recipients also pass the acid/enzyme rich stomach to then act systemically **[37,38].** Thus, such an exosome subset is also able to resist the similar acid/enzyme digestion of the phagolysosome microenvironment [**69,70,71,72,73],** to subsequently serve as Ag-specific immunogens to drive COVID-Ag-specific non-protective just reactive immune responses that underly LHS.

Conclusion: Residual stimulating COVID viral Ag that can induce the non-infectious immunologic LHS can be peptide COVID-Ag/MHC-specific exosomes or Ab FLC-coated Ag-specific exosomes associated with the remains of NETs, or stably present in phagolysosomes of APC.

Our hypothesis is that the in CPT the plasma contains billions of convalescent exosomes mediating a suppressive resistance to protection by the companion beneficial hyper-immune convalescent anti-COVID-19 Ab

We propose that the immunologic aspects of the infection, due to its Ag-inducing immune cell-derived exosomes, that are often present in the convalescent plasma, have negative suppressive effects on the developing host protective cell mediated immunologic responses developing early in the course of the viral illness when CPT is given to active infected patients, thereby interfering with the potential benefits of the convalescent hyper immune Ab also present in the therapeutic plasma from surviving COVID-19 patients.

We further propose that among these negative-acting exosomes some are Suppressor T cell-derived and are antigen (Ag)-specific for the COVID-19 virus, and further that this exosome Ag-specificity is due to antibody (Ab) free light chains (FLC) expressed on the surface of the involved inhibitory exosomes. These suppressive COVID-Ag-specific exosomes are postulated to be part of a multi-cellular circuit consisting of immunosuppressive T cells, collaborating B cells and macrophage APC, as has been shown to occur in an appropriate mouse model that we have established **(Fig. 1).** The created model features high dose Ag tolerance that is designed to imitate the situation of acute high dose foreign Ag exposure occurring during such a viral infection in the absence of the changes that infection might induce **[36,37].**

These proposed ideas are generated by a murine immunologic tolerance model recapitulating systemic high Ag dose exposure of viral infection

Such effects outlined above are seen in the murine model of high Ag dose immunologic tolerance designed to recapitulate viral Ag exposure over time in the absence of active infection. Results in the model have been published and repeatedly show that systemic administration of high Ag doses, employing a variety of Ag, are able to induce $CD8^{pos}$ Ag-specific suppressor T cells. These high Ag dose tolerance induced $CD8^{pos}$ Ag-specific suppressor T cells are not related to Treg cells as they operate in the absence of FOXP3 transcription factor that binds the genetic DNA promoters for genes involved in the function of T regulatory cells (Treg) [**36,37].**

Instead, these particular $CD8^{pos}$ Ag-specific suppressor T cells produce strongly inhibitory exosomes that have become coated with Ab FLC produced by companion immune B1a cells stimulated by the extensive Ag exposure, to account for their Ag specificity through endogenous processes of the actively immunized high Ag dose tolerized murine hosts **[36],[37].** Additionally, and in separate experiments, such Ag-specific Ab FLC can be placed on the surface of activated exosomes from similar Ag tolerized immunoglobulin deficient animals through mere in vitro incubation at 37^{o} C with monoclonal Ab FLC of chosen Ag-specificity [**36,37,38].**

Crucially, the suppressor T cell-derived exosomes additionally deliver in particular miRNA-150 that in this instance is inhibitory of targeted acceptor Ag-specific Ag/MHC-surface expressing macrophages that are acting as APC [**30].** This Ag-specificity proceeds by the Ab FLC on the surface of the T cell-derived exosomes binding to the specific Ag peptide complexed in MHC on the surface of these macrophages acting as APC [**38]**. These primary-acting Ag-specific Ab FLC coated $CD8^{pos}$ T cell-derived suppressive exosomes have been shown to be able to transfer their carried specific inhibitory miRNA-150 to these targeted APC to then alter their functional abilities in a suppressive direction, as

demonstrated both in vivo and in vitro [**36,46].** Their suppression occurs through transfer of inhibitory miRNA-150 associated with these primary Ag-specific exosomes and generated endogenously from the active high-Ag-dose-tolerogenesis imitating high dose viral Ag exposure in the murine donors, or experimentally by ex vivo associated by in vitro 37^{o} C incubation with selected commercial engineered miRNA-150 **[36,46].**

As noted, the targeting of the APC by the T cell-derived Ag-specific exosomes is via the CD8pos T cell-derived suppressive exosome surface Ab FLC that bind to the Ag peptide complexed in MHC on the surface of the targeted macrophage APC [**30,38].** For the multicellular suppressor circuit that is active here, the primary-acting suppressor T cell-derived Ag-specific exosomes thus alter the targeted macrophage APC towards suppressive function that induces them to release secondary suppressive exosomes **[74] (Fig. 1).** These downstream APC-derived secondary inhibitory exosomes have surface Ag peptide/MHC complexes enabling them to proceed to transfer miRNA different from miRNA-150 to the final targeted Ag/MHC-specific $\alpha\beta$-TCR positive effector T cells at the immune synapse. Therefore, this macrophage APC surface Ag/MHC specificity allows these secondary suppressive APC-derived exosomes to bind to the surface $\alpha\beta$-TCR of particular Ag/peptide-specific companion effector $\alpha\beta$-T cells at the immune synapse to suppress their function by transfer of a different inhibitory miRNA [**30,74] (Fig. 1).**

In actively viral infected COVID-19 patients that resist the benefits of CPT, we postulate that the high Ag dose experience of the viral infection in the donors of the convalescent plasma may have triggered a similar suppressive circuit that features Ag-specific inhibitory exosomes that are present in the plasma used for CPT. In this case, the suppressive Ag-specific exosomes would be coated with anti-COVID-19 Ag-specific Ab able to inhibit newly developing APC in the actively infected recipients. Also, the convalescent plasma might also contain analogs of the secondary suppressive

macrophage-derived COVID peptide Ag/MHC-surface specific exosomes and these high Ag dose induced Ag-specifically-acting exosomes in the convalescent plasma similarly can inhibit the required early developing anti-viral-peptide/MHC-specific cytotoxic $CD8^{pos}$ $\alpha\beta$-T cells in the recipients of CPT that are needed for resistance to the virus, and also inhibit their INF-γ producing and perhaps also helper anti-COVID-19 Ag-peptide/MHC-specific $CD4^{pos}$ T cells needed guide B cell production of convalescent hyper immune Ab. Thus, all together these suppressive Ag-specific exosomes resulting from high dose COVID-19 viral Ag induced tolerogenesis and present in the donor plasma eventually used for CPT, would therefore by their suppressive effects, antagonize what anti-infection help can be provided by the companion anti- COVID-19 IgG hyper-immune Ab in the convalescent plasma.

Conclusion: A murine model designed to mimics the chronic high dose COVID Ag exposure during active viral infection shows induction of convalescent immunosuppressive exosomes in the plasma used for CPT that inhibit the function of APC and effector ab-T cells just developing during early COVID-19 viral infection by transfer of inhibitory miRNAs that antagonizes the beneficial effects of the plasma hyper immune convalescent Ab. .

FIG. 1

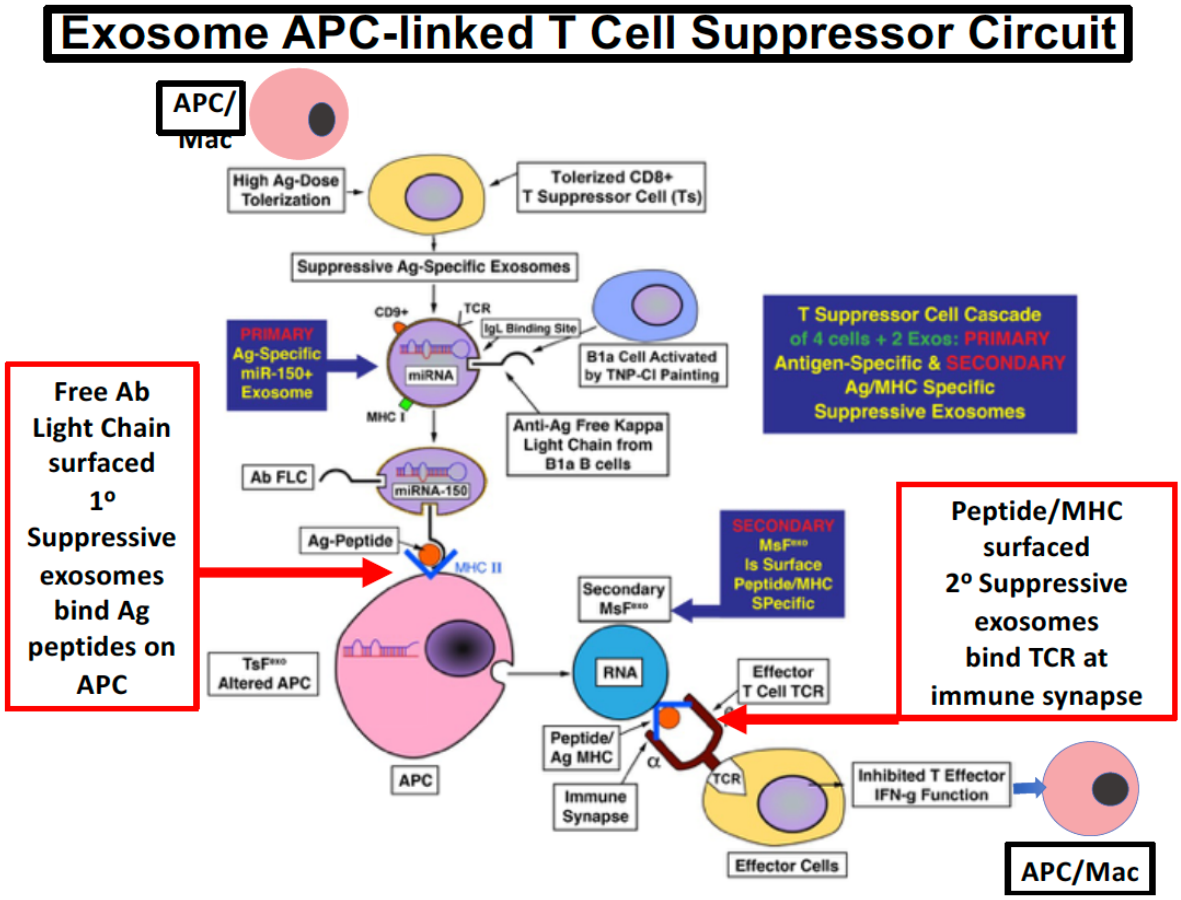


Fig. 1. Legend

Exosome APC-linked T Suppressor Circuit in Convalescent Plasma;

Demonstration of the Primary and Secondart Varieties of Ag-specific Exosomes.

Recipients are treated with high systemic doses of Ag over time to mimic exposure to the viral Ag during acute COVID-19 infection. This induces in the plasma suppressive primary exosomes derived from CD8-pos non-Treg T cells that become Ag-specific by surface expression of Ab free light chains

derived from companion B1a B cells that also were activated by excessive viral Ag. The Ab binds Ag-peptides in MHC on the surface of APCs to alter them epigenetically via transfer of miRNA-150. These fuctionally altered APC then can produce secondary suppressive exosomes with surface expression of the COVID-19-peptide/MHC complexes that can conjugate bind to the $\alpha\beta$-TCR of effector T cells at the immune synapse to suppress their functions. This results in transfer of other miRNA to inhibit effector T cell production of INF-γ and other tissue altering cytokines.

What are the possibly inhibitory convalescence exosomes that might counteract the long hauler syndromes?

In a word, in convalescent plasma there is a collision between desirable viral resistance promoting developed antibodies and undesirable convalescent cellular immune suppressing exosomes. These inhibiting exosomes are appropriate to convalescence, but when given early in infection would interfere with endogenous early developing profitable T cell mediated anti-viral responses.

At the least, there are the two varieties of Ag-specific exosomes that are generated during convalescence via the excessive viral Ag exposure of the preceding severe infection. Now in convalescence they are attempting to suppress cellular immune inflammatory processes that have run wild in active COVOD-19 infections. However, administering these exosomes early in the infection during the development of the protective immune responses, would work in an antagonistic direction compared to the beneficial hyper immune antibodies by suppressing nascent attempts of the cellular immune system to react profitably to the infection.

Firstly, there are those late developing Ag-specific exosomes; potentially produced by the inciting excessive viral Ag environment that are induced in suppressor $CD8^{pos}$ T cells and are coated with specific anti-COVID-19 Ab FLC. They act by binding to COVID Ag peptides on the surface of

monocyte-macrophage-DC lineage APC by transferring to the APC suppressive likely miRNA-150 to mediate epigenetic alterations in these targeted cells. In the case of administration of the convalescent plasma early in the infection, the result likely will be to inhibit the potential participation of these APC in their diverse participation in various endogenous Ag-dependent protective aspects of the processes developing during the early acute infection.

Secondly, there are the other main variety of Ag-specific exosomes derived from cells of the activated monocyte-macrophage-DC lineage that express surface Ag-specific complexes composed of COVID-19 specific Ag-peptides complexed with host MHC. These similarly are late generated via the excessive viral Ag exposure of the acute severe infection to now in convalescence to finally thwart the positive immune inflammatory processes that have run wild in active COVID-19 infections, at the level of the effector T cells. However, when given early in the infection when the developed convalescent antibodies likely will be helpful, these Ag-/MHC-specific suppressive exosomes will thwart the nascent positive-acting effector T cells of early infection, that are just beginning to combat the deleterious aspects of the acute viral infection. The macrophage APC targeted by the Ab coated Ag-specific exosomes in convalescence to produce inhibitory monocyte/macrophage cytokines and transfer yet other and suppressive miRNAs to inhibit COVID-19 viral-specific $\alpha\beta$-TCR expressing effector T cells. Early in infection when CPT is given these will just be beginning attempting to produce profitable protective cellular immunity of active COVID-19 viral infection. Acting now early in infection to down-regulate activation of numerous protective T cells and T cell-dependent ancillary cell responses is contrary to the effects that it is hoped that the convalescent hyperimmune Ab will provide.

Conclusion: In convalescent plasma there is a collision between desirable viral resistance promoting developed antibodies and undesirable convalescent cellular immune suppressing exosomes. These inhibiting exosomes are appropriate to convalescence, but when given early in infection would interfere with endogenous early developing profitable T cell mediated anti-viral responses.

Patients with Long Haulers Syndromes (LHS) consisting of serious illnesses after apparently surviving the acute infections, that may be mediated by remaining Ag-specific and Ag-responding exosomes

Here we hypothesize that in some patients following survival of clinical COVID-19 infectious illness, there is retention of whole Ag or Ag fragments of the COVID-19 virus, or aspects of COVID-19 Ag, that are able to drive anti-COVID-19 *non-protective* immune inflammatory responses that play a significant role in the auto-immune-like illnesses of these patients with LHS. As noted, we do not designate these as post-COVID because in many patients the clinical LHS seems to be just a continuation of aspects the acute illnesses. Thus, most of the symptoms and signs have been similar to the those developed during the acute phase of the infectious phase of the COVID-19 illnesses, but not necessarily. Note that the vast majority of long haulers test negative for COVID-19 viral genetic material. Thus, the protective aspects of the immune response like the essential anti-COVID-19 Ag-specific $CD8^{pos}$ cytotoxic anti-viral killer T cells and hyper immune Ab have won the day. However, immune inflammatory responses to retained Ag of the viral agent persist.

This is analogous to the recent finding that glycoprotein of Borellial spirochete inciting bacterial organisms that can persist to drive post infection Lyme Disease arthritis in 95% of patients [**75**,**76]** instead of the molecular mimicry postulates, that are now considered antique and less likely **[77,78].** Others have suggested that the LHS are relapses of continued infection via protection of the infecting organism in released SARS-CoV-2-loaded exosomes and/or other EVs **[79]** However, these patients no longer have fevers that are found in >90% of active infections and we think that the strongest evidence points to cessation of active infection and continuation of immune reactivity. Accordingly, although the number of ACE2 receptor-binding domain (RBD) of the spike protein Ag of virus-specific memory B cells remains unchanged 6 months after infection are undergoing clonal turnover **[79a].** Thus, their produced antibodies have greater somatic hypermutation, resistance to

RBD mutations and increased potency, indicative of continued evolution of the humoral response. As potential stimulant, intestinal biopsies obtained from asymptomatic individuals at 4 months after the onset of COVID-19 disease revealed persistence of immunoreactivity in the small bowel, while nasopharyngeal-swab PCR assays were negative for virus at the time of biopsy **[79a].** This suggests that the memory B cell response to the virus evolves over 6 months after infection in a manner that is consistent with antigen persistence.

Besides retention of relevant non-infectious viral Ag or viral Ag fragments; possibly in an Ag-presenting mode like in Ag peptide/MHC-surface specific mini-APC exosomes, driving these pathogenic immune responses following COVID-19 infections, there are other routes to retained Ag-specificity to promote immune inflammatory responses in patients with LHS. It is possible that there developed anti-idiotype Ab to Ag combining sites of anti- COVID-19 IgG Ab. For the immune system, these configurations can imitate specific COVID-19 Ag [**80],** or there may occur an analogous process inducing new COVID-19 Ag mimicking immune responses to the COVID-19 peptide Ag/MHC-specific combining idiotype sites of anti-COVID-19 $\alpha\beta$-TCR [**81].** A third possibility for generating resulting COVID-19 Ag-specific immunopathogenic exosomes participating in the LHS is that anti-self Ag-specific responses were generated via molecular mimicry or the like as postulated previously in many autoimmune diseases, but as mentioned now thought to not be relevant [**77,78].**

Whatever the inducing Ag-specific stimulus it is postulated that part of the generated clinically deleterious immune inflammatory responses in patients with LHS, that are beyond protective immune responses, is generation of Ag-specific Ab coated immune exosomes that can play a significant role in these processes. Such Ag-specific exosomes may be derived from some of the numerous subsets of COVID-19 viral induced immune T cells or B cells [**36,38].**

As noted, such anti-COVID-19 Ab FLC coated Ag-specific exosomes derived from $CD8^{pos}$ suppressor T cells have been demonstrated previously [**36,37],** as have other Ag-specific Ab FLC coated exosomes generated from B1a B cells as well [**38].** Other prior work has described mast cells coated with the Ig Ab derived FLC **[39,40,41,42,43,44,45]**. Yet other human studies have described the binding of Ab FLC to a variety of leukocyte types, with the highest level of binding being demonstrated on monocytes, but also onto human T cells, B cells and NK cells [**82],** perhaps through binding in part to cell surface sphingomyelins [**83].**

Clinical problems in COVID-19 patients with Long Hauler Syndromes

Clinical syndromes in LHS patients seem to possibly involve almost all organ systems in which immune effector exosomes and Ag-specific exosome participation can be proposed. Approximately 10-20% of people experience prolonged illness after recovery from acute Covid-19 infections**.** Thus, LHS constitute multisystem diseases, sometimes occurring even after a relatively mild acute illness. The symptoms of LHS can be fairly similar to what people experience in the acute phase of the illness, but typically they are not as severe. [**84,85,86,87].**

Still quite common have been joint pains and persistent difficulties in taste and smell. Many major diseases particularly developed during acute COVID-19, like stroke and diabetes also can go on along with problems developing out of what were non-specific effects of the acute viral illness, such as diarrhea, hair loss, tinnitus, sore tongue, rashes and night sweats**.** Common persisting measurable increased laboratory levels include: serum ferritin (8% of LHS patients), C-reactive protein (CRP) (8%), cardiac natriuretic peptide (11%), fibrin D-dimer degradation product (20%), procalcitonin (4%) and IL-6 (3%) **[84,85,86,87].** Remarkably, more than half of those affected had not recovered by three months [**88].** In fact, in followed health care workers, LHS can be present in a small minority even eight months after acute COVID-19 illness [**89].**

Organ damage caused by COVID-19 viral Ag that persist in LHS

Of course symptoms related to the lung disease are common and include: persistent cough, chest discomfort, shortness of breath, reduced pulmonary diffusing capacity, sleep apnea, and pulmonary fibrosis. Abnormal chest X-Rays and or computed tomography (CT) lung scan abnormalities also have been identified to persist [**90].** It is established that the type of pneumonia often associated with COVID-19 can cause long-standing damage to the alveoli so that resulting scar tissue can lead to long-term breathing problems. However, it should be pointed out that in pneumonia due classically to bacteria [**91],** like COVID-19 viral infections [**92**], can have long persisting X-ray and CT abnormalities can after diminution of the acute infectious diseases.

Although clinical COVID-19 disease primarily affects the lungs, it can damage many other organs as well since there are effects on endothelium of vessels, resulting in necrosis and clots **[93].** Resulting organ damage may increase the risk of long-term health problems in these Long hauler patients. Other organs that may be affected by COVID and persist in LHS prominently include heart problems. These cardiovascular sequelae include rapid or pounding heartbeat with arrhythmias and when severe even myocarditis. Thus, cardiac imaging tests taken months after recovery from COVID-19 infections have shown lasting damage to the heart muscle, even in people who experienced only mild COVID-19 symptoms [**94].** This may increase the risk of future heart failure or other cardiac complications. Current registered NIH clinical studies of these aspects are called “Cardiopulmonary Inflammation and Multi-System Imaging During the Clinical Course of COVID-19 Infection in Asymptomatic and Symptomatic Persons.”

Further there are possible thrombotic problems with a tendency for persisting blood clots, since the COVID-19 virus makes blood cells more likely to aggregate, clump and form clots. While large clots

can cause heart attacks and strokes, much of the heart damage caused by COVID-19 is believed to stem from very small clots that block capillaries in the heart muscle [**95].** Other parts of the body that can be affected by blood clots include the lungs, legs, liver and kidneys. COVID-19 can also weaken blood vessels and cause them to leak, that can contribute to potentially long-lasting problems with the liver and kidneys [**96].**

Central nervous system (CNS) aspects of COVID-19 LHS: Chronic Fatigue Syndrome

Regarding effects on the CNS, the acquired fatigue sometimes is very severe like the profound fatigue occurring in clinical mononucleosis. Thus, suggested Chronic Fatigue Syndrome (CFS) is in fact the most significant symptom that is being seen across the board in COVID-19 long-haulers. As in other instances this group often feels very run down and tired. They cannot exercise or merely exert themselves to perform simple tasks, like walking to the mailbox, that will often leave them exhausted. Such chronic fatigue can be incredibly debilitating and frustrating. Symptoms include: overall incidence of 34%, depression/anxiety (42%) “brain fog” (81%), headache (68%), numbness/tingling (60%), dysgeusia (59%), anosmia (55%), and myalgias (55%) [**97].**

As noted, the brain fog and fatigue of Long Haulers are suggestive of CFS, that is now also called Myalgic Encephalomyelitis (ME/CFS) [**98].** Before this COVID-19 pandemic, many people with ME/CFS report that these debilitating symptoms began with what appeared to be an infection, particularly viral infections; certainly often after infectious mononucleosis caused by Epstein-Barr virus [**99].** Note that ME/CFS in the diagnosis codes for the International Classification of Diseases even calls the condition “post-viral fatigue syndrome”. In contrast to the diverse illnesses of COVID-19, LHS the main syndrome following the SARS coronavirus lesser pandemic in 2003 and 2004 was in the CNS with frequent Chronic Fatigue Syndrome (CFS).

Besides severe incapacitating fatigue, the symptoms observed in post-COVID-19 LHS patients, that resemble parts of CFS include: unexplained pains, neurocognitive disability, compromised sleep, and unaccountable worsening of global symptoms following even minor increases in physical and/or cognitive activity [**100]**. Symptoms suggestive of autonomic dysfunction, as in other instances of CFS have been suggested, but some studies dispute this aspect pertaining to CFS of COVID-19 **[101].**

Thus, several neuropsychiatric symptoms have been reported in patients exhibiting LHS consisting of: headache (44% of the LHS patients), attention disorder (27%), and anosmia (21%). Other related. symptoms have been reported, that were not included previously, including brain fog and neuropathy **[100].** The etiology of neuropsychiatric symptoms in COVID-19 patients of course is complex and multifactorial. They could be related to the direct effect of the infection or severe hypoxia indued by the severe lung pathology **[102],** cerebrovascular disease (including hypercoagulation) **[100],** side effects of medications, and social aspects of having a potentially fatal illness **[100].** Adults have a double risk of being newly diagnosed with a psychiatric disorder after the COVID-19 diagnosis; with the most common psychiatric conditions that have presented being anxiety disorders, insomnia, and dementia. Sleep disturbances might contribute to the presentation of the psychiatric disorders **[100].**

Prompt diagnosis and intervention of appropriate neuropsychiatric care is recommended for all patients recovering from COVID-19. An increased attention to mental problems in hospitals and communities is needed during and after the COVID-19 pandemic. Hair loss after COVID-19 could be considered as telogen effluvium, that is defined by diffuse hair loss after an important systemic stressor or infection. It is caused because of premature follicular transitions from active growth phase

(anagen) to resting phase (telogen). It is a self-limiting condition that lasts approximately 3 months **[103],** that can cause emotional distress that synergizes with the CFS aspects.

Strong generation of Neutrophil Extracellular Traps from hyper-activated polymorphonuclear [PMN] cells in COVID-19

Neutrophil Extracellular Traps (NETs) are networks of extracellular DNA fibers released from activated neutrophils. NETs were originally described as positive-acting networks of extracellular DNA fibers released from activated neutrophils, that bind bacterial pathogens to aid in clearance of infections **[104].** These DNA-rich chromatin fibers are bound to neutrophil derived antimicrobial proteins that mediate an efficient extracellular system for killing of pathogens. They operated in addition to the usual intracellular phagolysosomal enzyme killing, and ordinarily this is controlled to minimize tissue damage.

Not only are NETs part of the response to bacteria, but also in responses pathogenic fungi such as *Candida albicans* **[105],** and similarly can respond to parasites like in *Plasmodium falciparum* infections **[106].** Further, and pertinent here, they are part of protective mechanisms in viral infections like in influenza **[107], [108],** and as a host defense response to Human Immunodeficiency Virus **[109].**

Alternatively, when control is lost and NETs then become excessive they can be pathologic. This can occur in a variety of cancers with tissue necrosis **[110],** and with sufficient inflammation in autoimmune diseases **[111],** and even allergy also can generate NETs **[112]**. A related example involving exosomes occurs in generalized pustular psoriasis, that is a rare severe inflammatory skin disease that can be life threatening **[113]**. The neutrophil : lymphocyte ratio in severe patients was higher than that in healthy controls and decreased after effective treatment. Neutrophils isolated from patients induced higher expressions of inflammatory genes in keratinocytes, including IL-β, IL-18, and

TNF-α, compared to normal neutrophils and secreted more exosomes than controls. These exosomes were rapidly internalized by the keratinocytes, leading to increasing expression of these inflammatory molecules via activating NF-κB and MAPK signaling pathways. The proteomic profiles in the neutrophil-derived exosomes accompanying NETS expressed pro autoimmune inflammatory and cell migration responses [**113].**

PMN activated by macrophage-derived exosomes in the cytokine storm are dominant in the pathology of severe COVID-19 viral infectious clinical syndromes

Systemic viral infections are usually accompanied by lymphocytosis, due to increase of $CD8^{pos}$ T cells that have αβ-TCR specific for the viral Ag that are responsible for killing viral infected cells . However, with COVID-19, there is a reduction in the number of lymphocytes, that may be due to increased production of strongly suppressive Transforming Growth Factor-β (TGF-β) and subsequent T cell exhaustion as some times seen in cancers, along with markedly increased neutrophil responses **[114].** Overly stimulated neutrophils profusely forming tissue damaging NETs constitute the distinctive pathology that is driven by a characteristic particular novel coronavirus associated hyperreactive uncontrolled innate immune response and features severely damaging neutrophils that can even lead to the high mortality of cytokine storm and ARDS **[115,116].**.

Neutrophilia and increased levels of neutrophil generating IL-8 and IL-6 are found in the blood of severe cases of COVID-19 and are associated with poor disease prognosis **[117].** In the many severe cases of COVID-19 diseases, deleterious NET chromatin DNA webs occur with released injurious neutrophil-derived microbicidal proteins including a variety of oxidant enzymes like myeloperoxidase, nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase), and nitric oxide synthase, as well as components with bactericidal activity such as neutrophil elastase, cathepsin, lactoferrin,

gelatinase and proteinases **[118].** As noted, formation of NETs in infections usually is a controlled process. It is conditional on the production of Reactive Oxygen Species (ROS) by the NADPH oxidase that usually can control unleashing effects of the numerous potential inflammatory constituents **[119].** However, in excess release of ROS at tissue sites, where there is instead harmful severe inflammation and induced microvascular thrombosis like in COVID-19 lungs of patients with ARDS, as also in severe sepsis **[120,121],** where formation of NETS becomes profound.

Further, in COVID-19 there is a correlation between NET generation and the inflammatory mediators of macrophage generated cytokine storm like TNF-α and IL-6 **[122]**. Moreover, new data on the role of NETs in the pathogenesis of COVID-19 disease indicates that this is a principal cause of the multi-organ dysfunction **[123].** With progression of the pneumonias to ARDS there is evasion of usual controls on innate immune responses, causing uncontrolled formation of NETs and consequent multi-organ failure. This extreme unchecked massive inflammation is thus due a harmful amplification loop between the inflammation and tissue damage induced by the dysregulation of NETs formation, called NETosis. Predominant in such NETosis is neutrophil elastase release, accelerating virus vascular entry inducing hypertension, thrombosis and vasculitis **[123].**

Thus, in severe COVID-19, neutrophils undergoing NETosis are the principal origin of released extracellular and circulating DNAs. A postmortem analysis of lung specimens from four patients who succumbed to COVID-19 and four patients who died from a COVID-19 of unrelated causes showed NETs in the lungs of each COVID-19 patient. NETs were found in the airways and in neutrophil-rich inflammatory areas of the interstitium, while NET-prone primed neutrophils also were present in arteriolar microthrombi **[124].** These results support the hypothesis that NETs may be drivers of severe pulmonary complications of COVID-19 diseases.

Among inflammatory cytokines engaged in the immunopathogenesis of COVID-19, IL-1β from activated macrophages is the key inducer of NETs. The opposite also can occur where the NETs

stimulate macrophages to increase the production of IL-1β, indicating a possible positive coupled pathogenic loop that may lead to excessive damage of the alveoli and pulmonary endothelium in patients with severe progression of COVID-19 **[125,126,127].** Accordingly, sera from patients with severe COVID-19, especially those intubated and requiring mechanical ventilation reflect these processes. They have elevated levels of DNA,), IL-β, myeloperoxidase (MPO) and citrullinated histone H3; the latter two being specific markers of IL-β stimulated NETs. The serum cell-free DNA levels strongly correlate with absolute neutrophil counts and levels of acute-phase C-reactive protein, D-dimer, lactate dehydrogenase and neutrophil elastase.

Most importantly, activators of these processes also are present in such sera that themselves can trigger in vitro NET release from control neutrophils **[128].** We favor the idea that these are NET-generating exosomes released by the heavily activated macrophages **[129],** that are the dominant source of cytokine storm constituents, or by the activated neutrophils **[130]** or platelets **[131]** of sepsis. Accordingly, miRNAs that are chiefly carried by exosomes are now recognized as new regulators of formation of NETs **[132].**

Quite relevant here, considering that in COVID-19 diseases the cytokines of cytokine storm are principally macrophage-derived **[133],** is the finding that exosomes derived from stimulated macrophages can induce NETs. These original studies were in atherosclerosis where it was shown that macrophages treated with activating lipids secreted exosomes carrying miR-146a that in particular induced neutrophil formation of NETs by overly increasing ROS production **[134]**. Thus, is seems likely that in severed COVID-19 infections the induced serum factors causing release of NETs in vitro from normal neutrophils are activated exosomes derived from the inordinately activated macrophages. However, we also note that activated platelets also can release exosomes and microvesicles that also can induce NET formation as can proinflammatory cytokine release relevant to coagulation disorder aspects of ARDS **[135].** Also, activated degranulating neutrophils themselves

release exosomes that can become coated with elastase producing a multivalent form of the enzyme with enhanced ability to degrade extracellular matrix thought relevant to chronic obstructive pulmonary disease **[136].** As exosomes and NETs are both extracellular, interactions between them are inevitable. In fact, induction of NETs by exosomes has been reported in cancer **[137],** and in sepsis **[131]**, , which of course is dominant in severe COVID-19, possibly with ARDS.

NETs facilitate antigen presentation by mediating adjuvant effects for remaining COVID-19 Ag that may persist to generate LHS

Here we are proposing that NETs in COVID-19 disease can serve an adjuvant-like effect for retained Ag, leading to Ag stimulation underlying Long Hauler Syndromes. Importantly, it has been determined that NETs play a crucial role in the adjuvant effects of commonly employed aluminum adjuvant **[138].** At the side of aluminum adjuvant injection, host DNA derived from NETs resulting from large irritative induced PMN infiltrative swarming act to enhance MHC class II-mediated Ag presentation by dendritic cells to prolong CD4 T cell interactions, promoting otherwise minimal antigen presentation by reducing their activation threshold **[139].** Furthermore, when PMN swarming is induced by an in vitro system of artificial microparticles, there is neutrophil release of exosomes that are found to have activated the neutrophils and contain the proinflammatory mediator galectin-3, suggesting that such EVs have an active role during neutrophil swarming **[140].**

Hypothesized immunopathogenesis of COVID-19 LHS involving COVID Ag-specific exosomes and the potentiating Ag-presenting effects of NETs

There are two potential scenarios. They involve two different Ag-specific exosomes acting at NETs to gain the adjuvant-like APC permitting functions of the NETs. In the first, Ag-specific exosome mini-APC from DC or neutrophils with surface residual COVID-19 peptide Ag/MHC surface complexes

bind to anti-COVID-19 peptide/MHC-specific αβ-TCR on effector T cells at the immune synapse. This results in transfer of positively acting miRNA that causes T cell release of pro-inflammatory cytokines and positive T cell help for anti-COVID B cells inducing LHS by mediating their production of anti-COVID-19. This occurs under the adjuvant-like APC permitting functions of the NETs.

For the second at NETs, Ag-specific T cell or B cell-derived exosomes coated with anti-COVID-19 Ab FLC bind to residual COVID-19 peptide complexed in MHC on APC from DC or neutrophils to deliver positive-acting miRNA. This induces APC production of secondary positive-acting exosomes with surface peptide/MHC complexes that bind to anti-COVID Ag/MHC αβ-TCR on effector T cells at the immune synapse. This results in effector T cell release of pro-inflammatory cytokines and positive help for anti-COVID B cells inducing aspects of LHS by mediating anti-COVID-19 Ab production. This occurs under the adjuvant-like APC permitting functions of the NETs.

Alternatively, it has been argued persuasively by others **[79]** that the crucial aspect is not the carry-on of Ag, but of virus in exosomes for a Trojan horse like situation; with late reappearance of infection driven LHS. This is interesting, but the predominant growing data is that true demonstration of live virus carry over is increasingly rare; but time will tell weather late effects are due to residual viral Ag as postulated here, or due to persistent live virus.

An intermediate hypothesis would be carry-on of viral encoding mRNA, perhaps in exosomes, translating into COVID-19, to then stimulate the immune system to drive the LHS analogous to the mRNA based Moderna and Pfizer vaccines translating into viral spike protein for development of protective immune responses.

How COVID-19 mRNA vaccine injections can possibly beneficially treat LHS?

Lastly, there is now are numerous anecdotal reports from patients of improvement in LHS when vaccinated with the mRNA based Moderna and Pfizer vaccines in about 10-20% of cases. This will

have to be established with proper control matched and randomized studies, but will be difficult with a need to quantitate diverse signs and symptoms in the great variety of LHS patient phenotypes. However, if vaccine treatments of LHS do produce documented improvement in the LHS clinical cases, then according to our formulation of `Ag-specific suppressive exosomes in CPT, then CPT therapy of LHS might be considered for producing even more effective treatment. This is because there would be a positive influence on multiple Ag-specific responses, beyond just to the viral spike protein produced by the mRNA based vaccines. If it is true that COVID-19 vaccines can be used for successful treatment of some patients with LHS, we hypothesize this represents a further potentiation of Ag induced tolerance, such that CPT using treatment with plasma that is selected from non-LHS fully recovered patients, can be a possible even more effective treatment for LHS.

In the first properly controlled study, some LHS COVID-19 patients say their symptoms are subsiding after getting vaccines

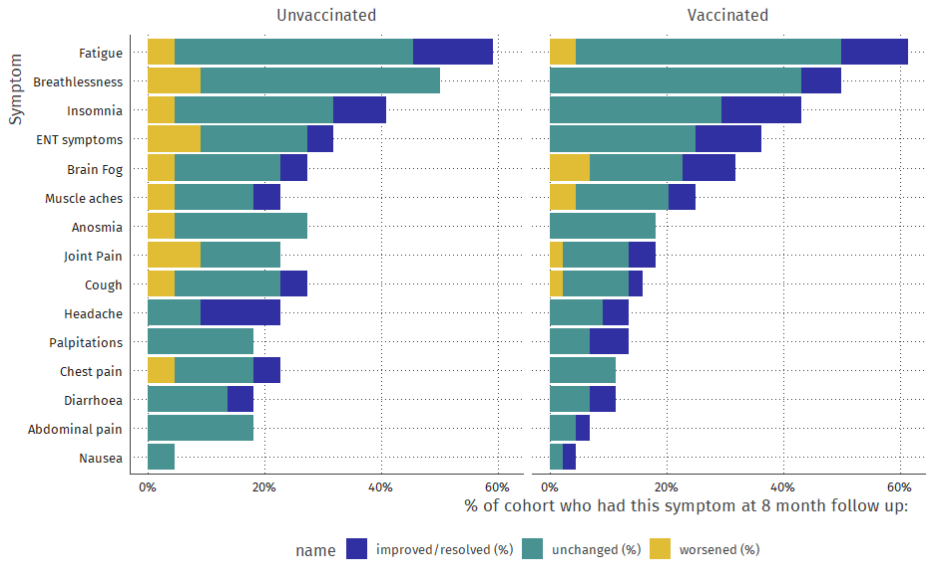
A study conducted by a team of scientists in Bristol, UK found there was “a small overall improvement” among Long Haulers after they received a COVID-19 vaccine. The study involved 44 vaccinated and 22 non-vaccinated patients with LHS **[141].**

This not yet peer-reviewed study, assessed patients in hospital with COVID-19 during the summer of 2020 at 3 and 8 months post-admission. Most of the LCS sufferers were highly symptomatic before vaccination, with predominating fatigue (61% of the LCS patients), breathlessness (50%) and insomnia (38%). Participants who received the Pfizer-BioNTech or Oxford-AstraZeneca vaccines between January and February were matched in terms of their symptoms at eight months with other patients from the same cohort who were not vaccinated. All were then reassessed one month after the vaccinated cohort received their first dose. Participants were telephoned to carry out quality of life questionnaires and were asked whether their individually evaluated symptoms had improved, stayed

the same, or worsened. The researchers found that those who had received a vaccine “had a small overall about 10% improvement in Long Covid symptoms”.

Thus, over a five month period there was a 5.6 % decrease in worsening symptoms and 23.2 % increase in symptom resolution among this vaccine group, compared to 14.2% and 15.4% for the unvaccinated control cohort with no difference identified between the Pfizer or AstraZeneca vaccines (i.e. with vaccination there was significant improvement and less worsening of symptoms). The research was limited by the small patient sample size, and it was acknowledged that “this is an initially hospitalized cohort so it cannot be directly extrapolated to individuals whose initial infection did not result in hospitalization” **[141].**

Figure 2: Symptoms at 8-month follow up with change following vaccination (or matched timepoint in unvaccinated group)



Conclusion: If LHS is due to residual Ag or even residual infection, convalescent COVID peptide-Ags/MHC exosomes cover many more Ag compared to mRNA vaccines that just encode COVID-19 spike protein.

SUMMARY

The hypothesis we are putting forward is that NETs play an important role in prolonging the non-anti-viral protective strictly Ag-reactive COVID-19 immune responses following active infection that characterize LHS. The NETs are postulated to act as adjuvants to enhance post infectious COVID-Ag-specific stimulated immune responses. This could feature the activities of Ag-specific mini-APC exosomes permitting further T cell priming via NETs and consequent continuation of pathogenic immune responses. Thereby, this association would link innate neutrophil and adaptive immune COVID-19 Ag-specific T cell responses underlying the LHS. They also could include a possible role for the other Ag-specific exosomes mediating processes dependent on their Ab-derived surface FLC that are able to bind COVID peptide Ag complexed in MHC on APC for subsequent transfer of miRNAs to prolong immune responses.

CONCLUSIONS:

Our overall hypotheses is that on recovery from COVID-19 active infections, minute residual quantities of viral Ag, or peptides associated with Ag presenting mini-APC Ag-specific exosomes, augmented by permissive adjuvant effects provided by the NETs, guide continuing immune T cell and B cell responses to mediate COVID-19 late LHS. In this case, therapy with COVID-19 convalescent plasma from recently completely recovered patients containing convalescent Ag-specific inhibitory exosomes is a reasonable choice for a trial for treatment of some patients with LHS. Hopefully, such LHS recipient patients with determined clinical improvement in well controlled studies would also have an easy objective blood test consisting of coupled solid laboratory data. Given the frequent occurrence of easy fatigue in patients with LHS and the know associations of peripheral blood exosomes with hypoxia **[142]** and with exercise **[143],** one possibility would be a clinical test of exercise induced peripheral blood changes that might include objective quantitative exosome markers of altered hypoxic associated function.

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Dear Colleagues

Per genesis of new therapy for LONG COVID with convalescent plasma from survivors, it is well known that there have been numerous very disappointing results of Convalescent Plasma Therapy (CPT) *in active infections with COVID-19*.

A question is how to account for this, given the huge history of *seeming* benefit of CPT in a variety of instances over more than the past 100 years.

We have published some reasoning *based on our experimental evidence* (Philip Askenase, **COVID-19 Therapy With Mesenchymal Stromal Cells (MSC) and Convalescent Plasma Likely Depend on Exosomes; Do the exosomes in convalescent plasma antagonize the weak immune antibodies? Editorial Review**, *Jo Extracellular Vesicles,* Volume10, Issue 1, October 2020,e12004, pages 1-19).

Here is the reasoning in sum:
In CPT there is a collision between desirable viral resistance promoting developed hyper immune antibodies and undesirable convalescent exosomes that act to suppress the “over the top ”cellular immunity of the acute infection by inhibiting antigen

presenting cells (APC) and cytokine producing effector T cells (our work, see attached power point presentation and references below).

These inhibiting exosomes are appropriate to convalescence, but when given early in infection may interfere with endogenous early developing profitable innate mononuclear APC and acquired-immune T cell mediated anti-viral IFN-g driven responses.

Knowing that there is growing interest in the Covid Long Haulers that go on with significant clinical syndromes after successfully dealing with the viral infection, I raise the possibility that CPT containing the potential of broadly Ag-specific suppressive exosomes, might be considered for possible effective treatment of the COVID-19 Long Hauler Syndromes. We argue that syndrome are due to residual COVID antigens perhaps complexed in remnants of neutrophil extracellular traps of the acute infection to which there is an aberrant immune response or remaining positive immune acting antigen specific exosomes The convalescent plasma inhibiting exosomes should certainly be superior compared the purported value of vaccines, as there would be an influence on multiple Ag-specific responses, beyond just to the viral spike protein solely brought by the vaccine.

The question for you is how to progress with this proposed point of view manuscript moving towards trials of LONG COVID treatment with convalescent plasma vs. normal plasma

Warmly

Phil A.

PS: Also attached below is the exosome part of my CV to show my expertise in this emerging area.

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PUBLICATIONS ON EXOSOME EXTRACELLULAR VESICLES IN IMMUNITY, NEURO INJURY,

CUTANEOUS INFLAMMATION, AND GENERAL BIOLOGY

ORIGINAL PEER REVIEWED PAPERS (numbers to the left are from my complete CV)

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Supplemental Figures for Review Only

POWER POINT SLIDE NUMBER

1. Method of administrating multiple high antigen (Ag) doses in mice, repeated over time, to mimic Ag exposure in a severe viral infection, induces suppressive CONVALESCENT PLASMA.

2. CONVALESCENT PLASMA-derived exosomes from mice treated with Ag high dose tolerization over time to imitate the immunology of viral infection are strongly suppressive of adoptive Th1 T cell immunity compared to normal plasma or plasma from sham treated animals (Groups E,F, & G).

3. Treatment with high Ag dose tolerized CD8+ T suppressor cell supernatant-derived anti-OVA CONVALESCENT EXOSOMES prior to Ag ear challenge on Day 4 of immunization is strongly suppressive (48-75%) of adoptive Th1 T cell immunity.

4. Method for determining in vivo active Delayed-Type Hypersensitivity ear swelling on day 4 of immunization that is suppressed by systemically injected high Ag dose tolerized CD8+ T suppressor cell supernatant-derived anti-OVA CONVALESCENT EXOSOMES, administered at the time of the 24 hr. maximum ear response.

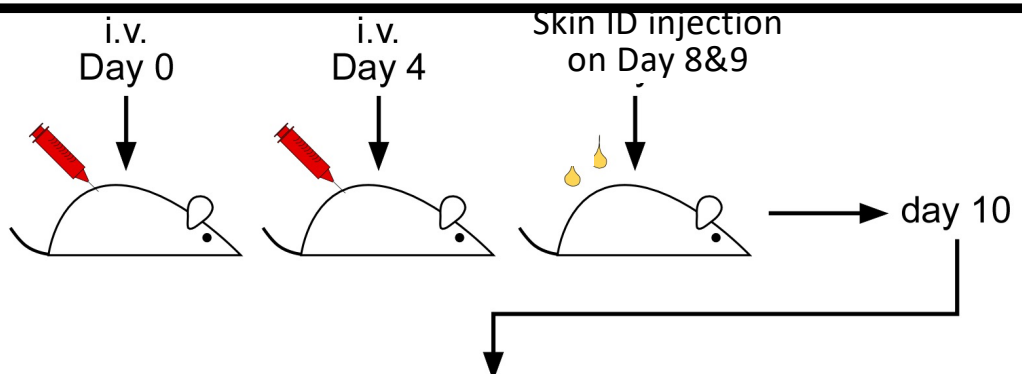
5. Determined in vivo active Delayed-Type Hypersensitivity (DTH) ear swelling on day 4 of immunization is suppressed 48-75% by systemically injected high Ag dose tolerized CD8+ T suppressor cell supernatant-derived anti-OVA CONVALESCENT EXOSOMES, administered at the time of the 24 hr. maximum ear response.

6. Treatment with Ts supernatant *anti-KLH* Ag-specific CONVALESCENT EXOSOMES of a differing Ag-specificity just after 24 hr. peak of the active OVA Ag ear swelling response at day 5 is non-suppressive. Thus, injected high Ag dose tolerized CD8+ T suppressor cell supernatant-derived anti-OVA CONVALESCENT EXOSOMES acted Ag-specifically.

7. Antigen-specific CONVALESCENT SUPPRESSOR EXOSOMES inhibit 24 hr. in vivo immune Th1 cell DTH ear skin swelling immune histologic inflammatory responses in adoptive immune cell recipients.

8. The CD8+ T cell-derived OVA Ag-specific miRNA-150pos CONVALESCENT SUPPRESSOR EXOSOMES use surface anti-OVA Ab to bind Ag peptide in MHC on the macrophage APC surface to induce their release of secondary miRNA-150neg CONVALESCENT SUPPRESSOR OVA-MsF-EXOSOMES inhibiting OTII anti-OVA effector DTH T cells at the $\alpha\beta$–TCR immune synapse (Group B vs. A), and their suppression is augmented by aggregating the exosomes with specific anti-OVA peptide-323 monoclonal antibody (Group B vs. C).

Multiple, Repeated Over Time, High Ag Doses to Mimic Ag Exposure in a Severe Viral Infection

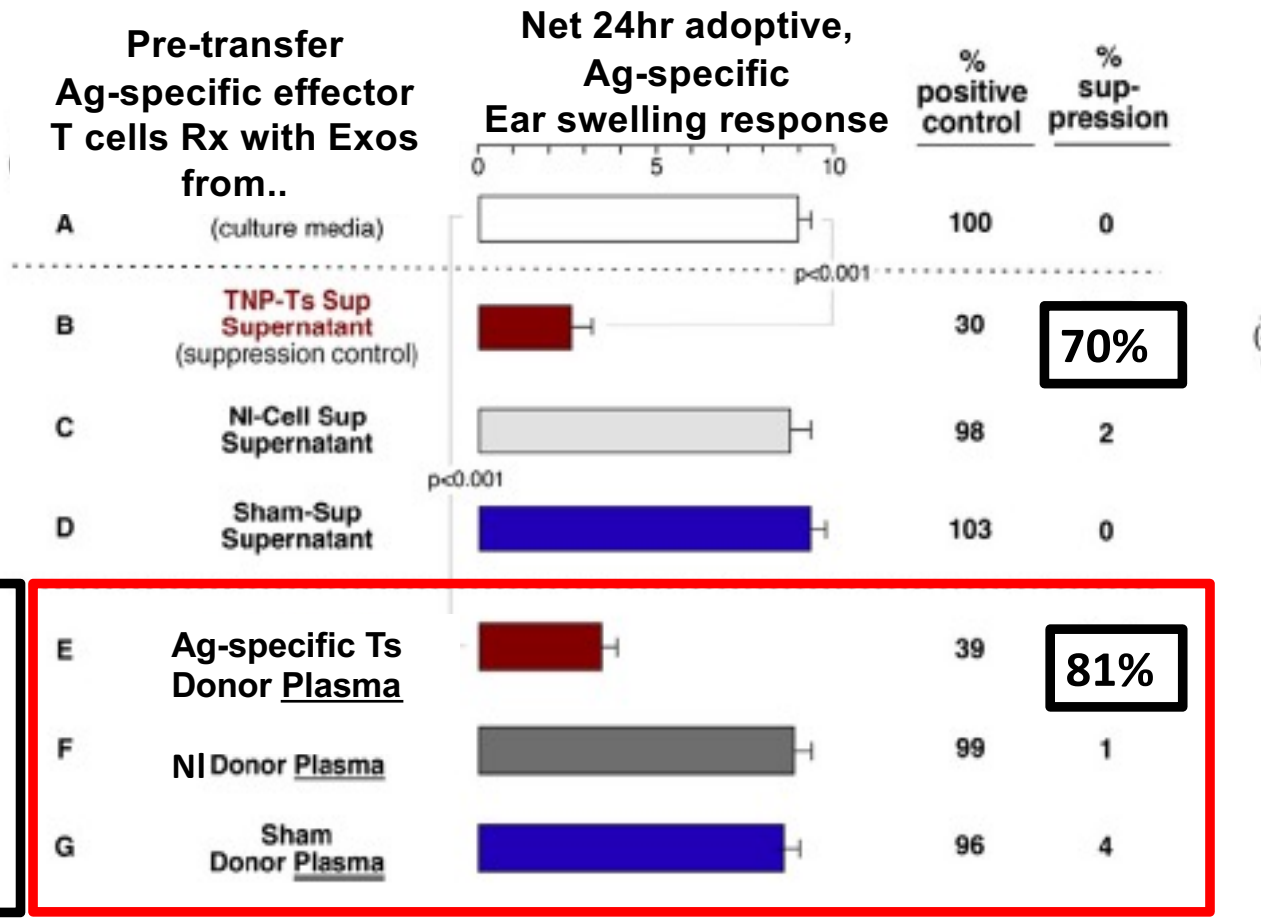


Harvest "CONVALESCENT PLASMA"

Test Ability to Modulate Classical In Vivo T effector Cell Mediated Skin Responses

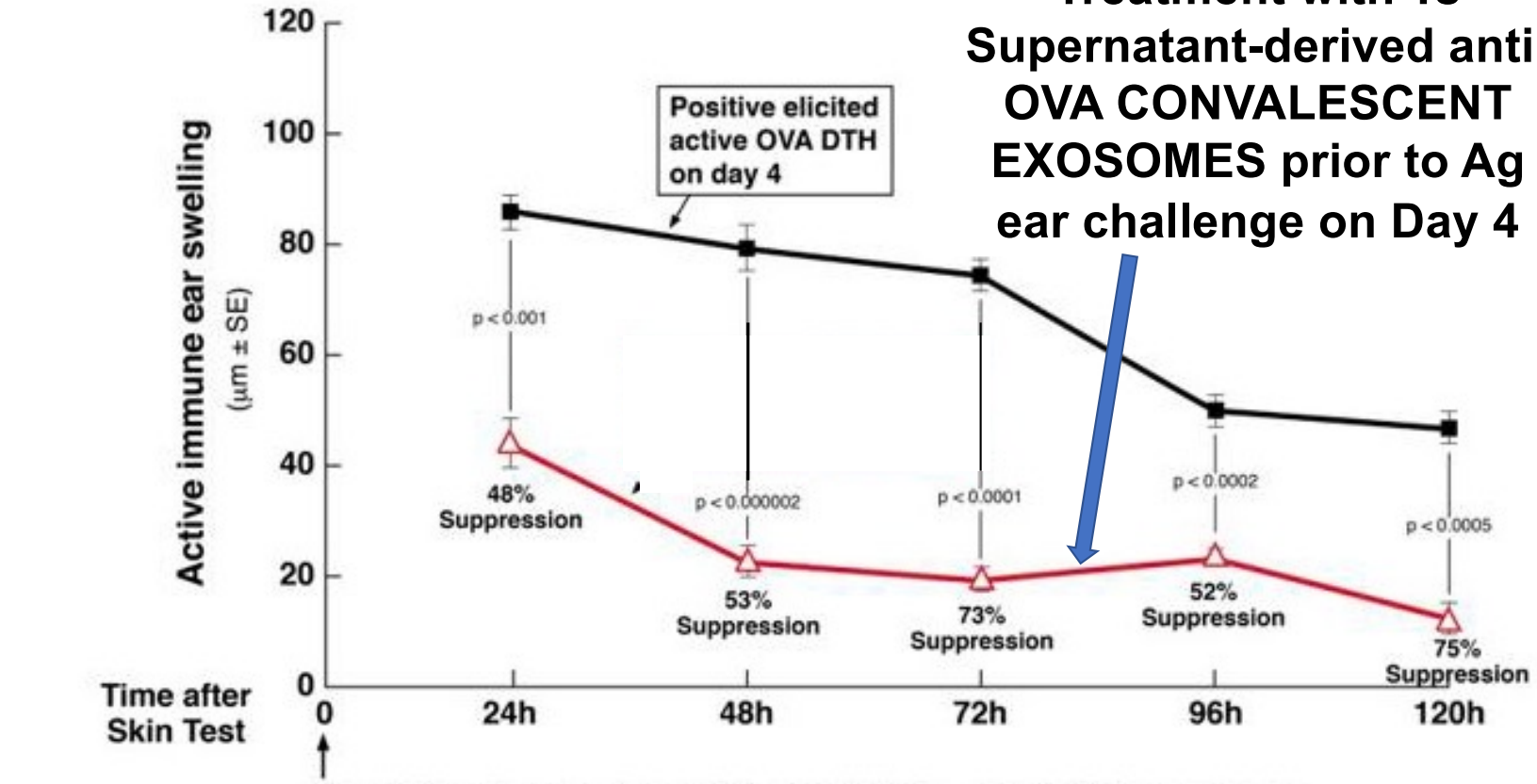
Process to Ag-Induced, Ag-specific Convalescent-Like Exosomes

CONVALESCENT PLASMA of mice specific Ag tolerized over time to imitate immunology of a viral infection

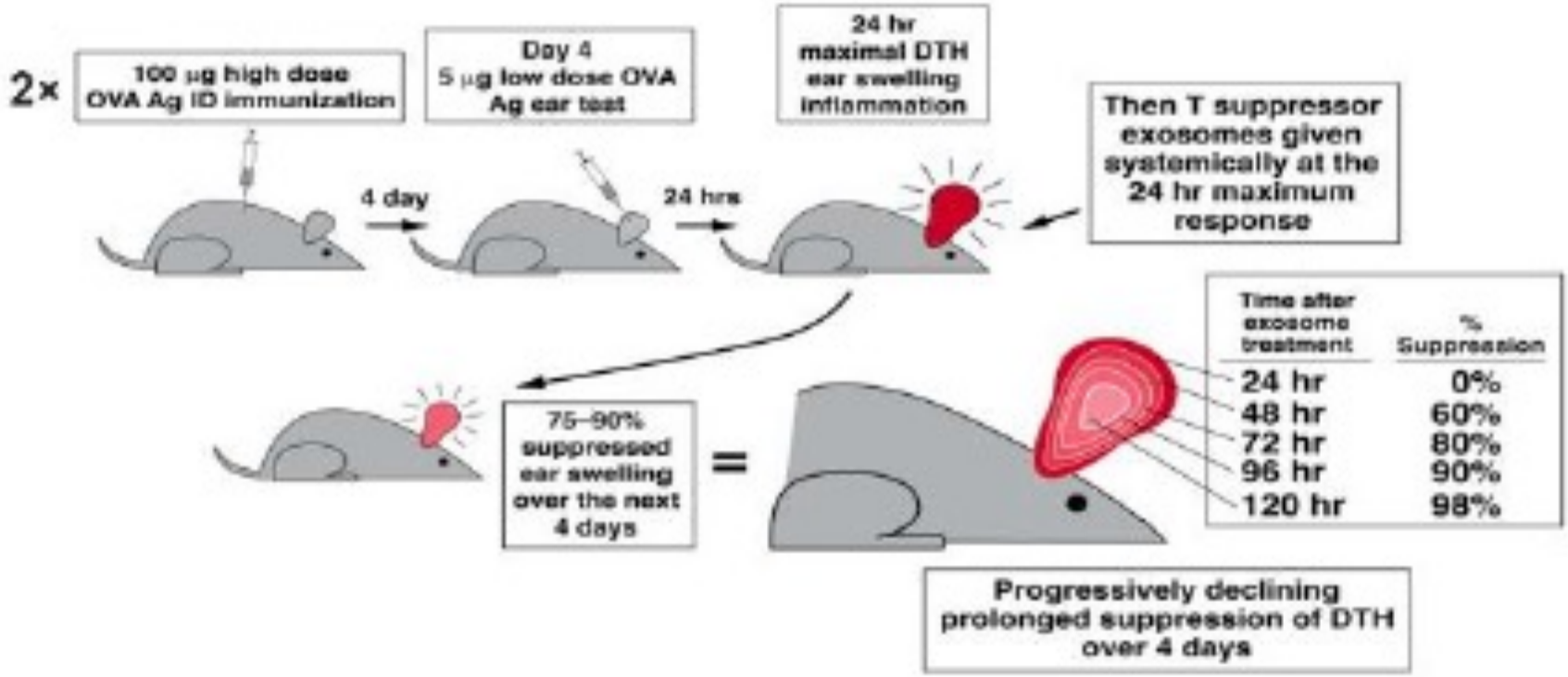


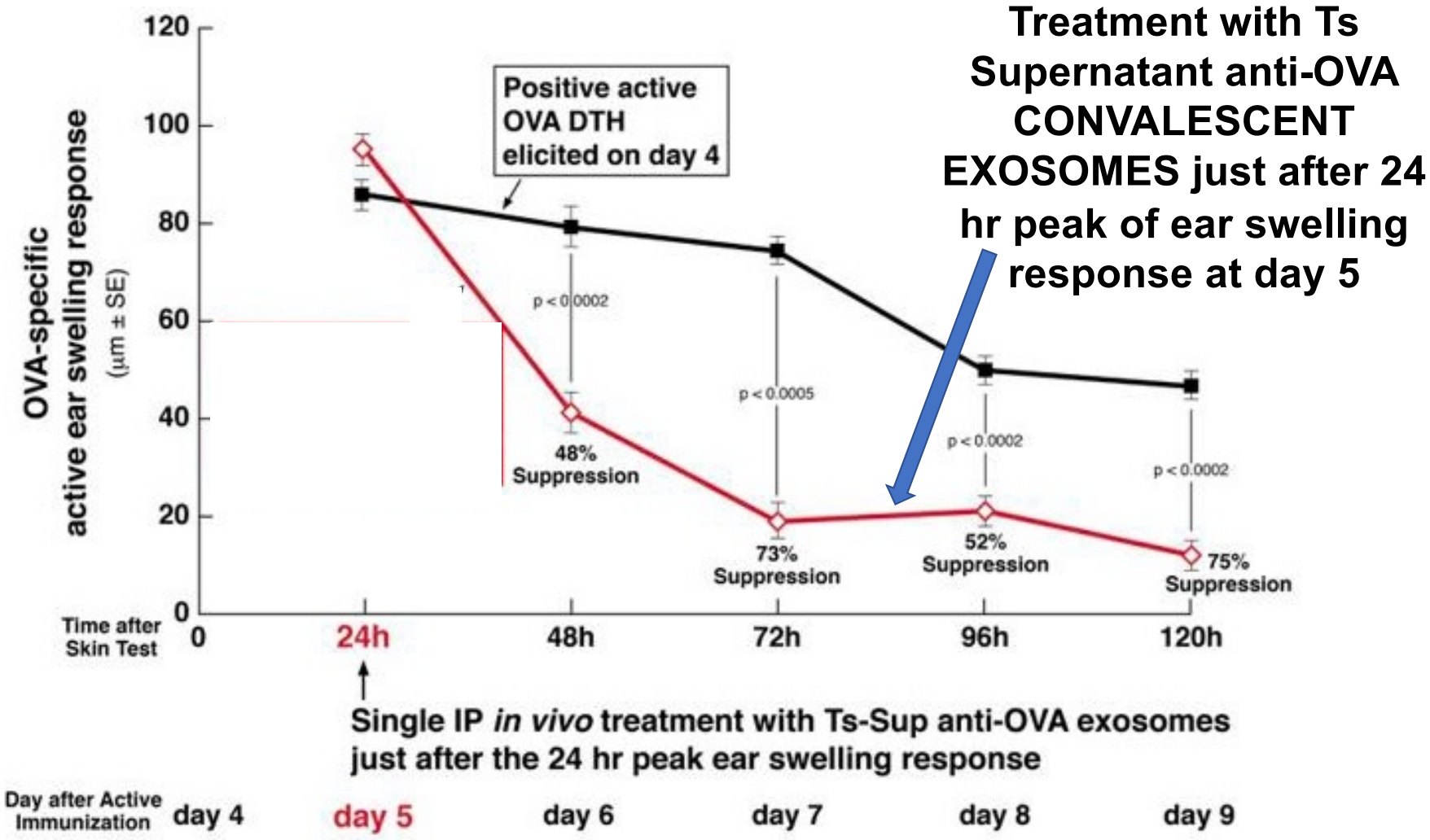
CONVALESCENT viral-like Ag-tolerized plasma suppresses effector T cell responses in skin.

Treatment with Ts Supernatant-derived anti-OVA CONVALESCENT EXOSOMES prior to Ag ear challenge on Day 4

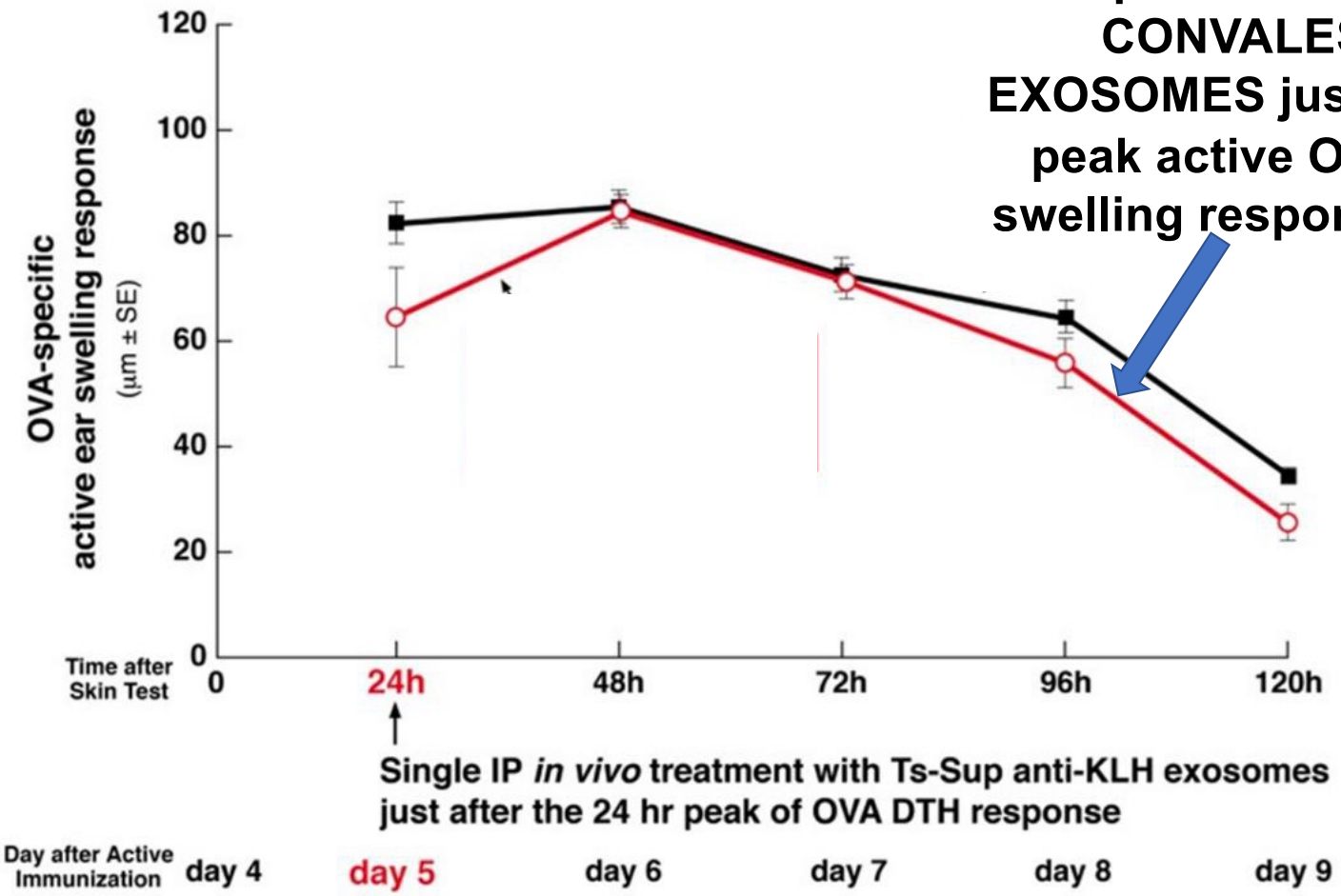


In Vivo active DTH is suppressed by systemically injected T suppressor exosomes, given at the 24 hr maximum ear response

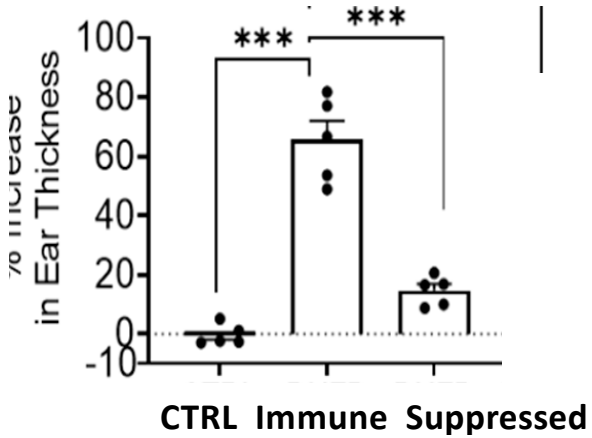



Treatment with Ts Supernatant anti-OVA CONVALESCENT EXOSOMES just after 24 hr peak of ear swelling response at day 5
OVA-specific active ear swelling response (μm ± SE)
120
100
80
60
40
20
0
Positive active OVA DTH elicited on day 4
p < 0.0002
p < 0.0005
p < 0.0002
p < 0.0002
48% Suppression
73% Suppression
52% Suppression
75% Suppression
Time after Skin Test
0
24h
48h
72h
96h
120h
Single IP *in vivo* treatment with Ts-Sup anti-OVA exosomes just after the 24 hr peak ear swelling response
Day after Active Immunization
day 4
day 5
day 6
day 7
day 8
day 9

Treatment with Ts Supernatant *anti-KLH* CONVALESCENT EXOSOMES just after 24 hr peak active OVA Ag ear swelling response at day 5

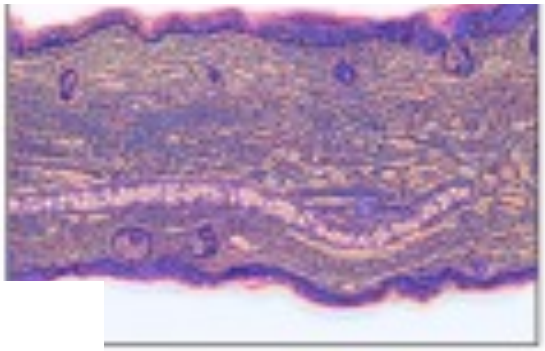


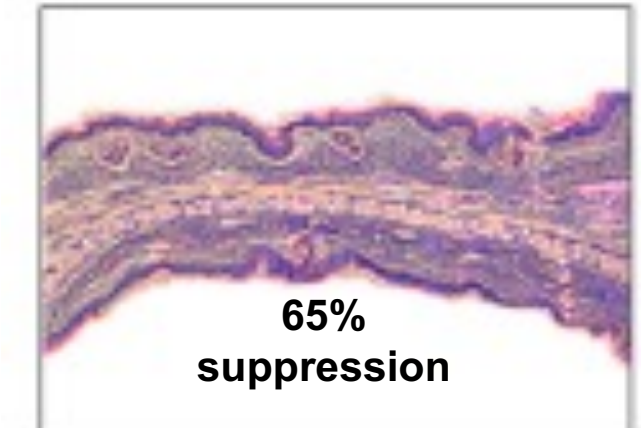
Antigen-specific CONVALESCENT SUPPRESSOR EXOSOMES inhibit 24hr in vivo immune cell DTH ear skin swelling responses in adoptive cell recipients



24hr non-immune Neg control mouse with + Ag ear challenge

24hr active pos control Ag-immune cell transfer with + Ag challenge ears of recipients





24hr adoptive DTH cells treated with suppressive CONVALESCENT EXOSOMES, then local Ag challenge of recipient ears

Suppressive activity of OVA-MsF^{exos} on OVA-DTH effector T cells is augmented by anti-OVA 323 peptide IgG antibody