Review

Cellular and Humoral Immunity and Infection Responses to SARS-CoV-2:

Immune Biomolecular Mechanisms by Case Study within SARS-CoV-2 Pathogenesis and Other Infections

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Abstract: The coronavirus 2019 (COVID-19) pandemic was caused by a positive sense single-stranded RNA (ssRNA) severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). However, other human coronaviruses (hCoVs) exist, of which Middle East Respiratory Syndrome (MERS) and SARS-CoV (SARS) showed higher mortality rates without causing a pandemic. As of December 2022, SARS-CoV-2 has resulted in over 6.6 million deaths worldwide through an array of acute to chronic pathologies. Historical pandemics include smallpox and influenza with efficacious therapeutics utilized to reduce overall disease burden. Therefore, immune system process analysis is required to compare innate and adaptive immune system interactions. Lymphatic system organs include bone marrow and thymus using a network of nodes throughout which white blood cells traverse glycolipid membranes utilizing cytokines and chemokine gradients that affect cell development, differentiation, proliferation, and migration processes as well as genetic factors affecting cell receptor expression.

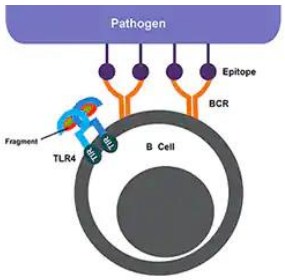

Figure 1: B Cell Recognition

Innate processes involve antigen-presenting cells and B lymphocyte cellular responses to pathogens relevant to other viral and bacterial infections but also in oncogenic diseases. Such processes utilize cluster of differentiation (CD) marker expression, major histocompatibility complexes (MHC), pleiotropic interleukins (IL) and chemokines. The adaptive immune system consists of Natural Killer (NK) and T cells. Other viruses are also contributory to cancer including human papillomavirus (cervical carcinoma), Epstein-Barr virus (EBV) (lymphoma), hepatitis B and C (hepatocellular carcinoma) and human T cell leukemia virus-1 (adult T-cell leukemia). Bacterial infections also increase the risk of developing cancer(e.g. H. pylori). Therefore, as the above factors can cause both morbidity and mortality along-side being transmitted within clinical and community settings, it is appropriate to now examine advances in single cell sequencing, FACS analysis and many other laboratory techniques that allow insights into discoveries of newer cell types. These developments offer improved clarity and understanding that over-lap with known autoimmune conditions that could be affected by innate B cell or T cell responses to SARS-CoV-2 infection. Thus, this review quantifies and outlines the nature of specific

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receptors and proteins relevant to clinical laboratories and medical research by documenting both innate and adaptive immune system cells within current coronavirus immunology case study data and other pathologies to date.

Keywords: COVID-19; B Cells; Neutrophils; T Cells; NK Cells; Innate; Adaptive; Cytokines; Chemokines; Adhesion Molecules; Antibody; Cluster of Differentiation; Receptors; Proteins; SARS-CoV-2; Serology

1. Introduction:

The causal virion SARS-COV-2 of the COVID-19 pandemic contains four immunogenic proteins composed of spike (S protein), nucleocapsid (N protein), envelope (E protein), and membrane (M protein) and associated subunits [1,2]. Current therapeutic development occurred before/after March 2020 when the World Health Organization (WHO) declared a pandemic. Technological advancement since 2017 has also allowed greater cellular phenotypic analysis and therefore it is clearer now that SARS-CoV-2 cellular protein s have different toles with M protein vital for assembly spike protein S for receptor entry, N protein but also E protein a potential pore forming protein [3,4]. Single cell RNA sequencing (scRNA-Seq), spectral flow cytometry (FACS) and mass cytometry (CyTOF) can detect markers enabling phenotypic analysis of all immune cell subsets [5–8]. SARS-CoV-2 infects cells via respiratory pathways and type II pneumocytes using angiotensin converting enzyme 2 (ACE-2) as the predominant receptor for entry [9] Disruption and infection of type II pneumocytes expressing ACE2 occurs through phospholipid membranes. Other receptors expressed on all leukocytes, platelets, and endothelial cells called cluster of differentiation markers (CD) include CD3, CD4. and CD19 amongst others used to classify immune cells. Other receptors currently implicated in initial SARS-CoV-2 cellular entry are type II transmembrane protease (TMPRSS2), asialoglycoprotein receptor-1 (ASGR1) and kringle containing transmembrane protein 1 (KREMEN1), dipeptidyl peptidase 4 (DPP4), neuropilin (NRP1) and CD147 [10–14]. Therefore, our research has focused on identifying all cellular receptor targets that can be either inhibited or stimulated to mitigate chronic diseases by targeting pleiotropic cytokines within immune systems with further detail.

Current vaccine antigens prime the immune system to recognize proteins via specific epitopes that can mutate thereby affecting immune cell recognition through B cell and T cell receptors (BCR/TCR). Prevention of chronic COVID-19 disease to pre-Omicron variants was estimated in these ratios within current vaccine immunogens and differential vaccine vectors employed by Pfizer/BioNTech, Astra Zeneca, Sinopharm and Novavax: BNT162b2:95.3%, AZD1222: 70.4%, BBIBP-CorV:79%. Immunogen development indicates NVX-CoV2373 at 72% when screened against Omicron BA.1 and BA4/BA5. Additional risk reduction of COVID-19 disease was estimated at 86%. Population studies showed variable SARS-CoV-2 protein antibody responses (76%:24% response/non-response). Estimated production of antibodies to S protein immunogens currently spans 6 months to 1.5 years. SARS-CoV-2 S protein mutations are now well documented in other studies to ascertain potential epitopes that affect the immune response [15]. Current spike specific B cell memory is estimated in response to vaccination, reaching 36% at 180 days (after 2 doses) with delayed antibody response to SARS-CoV-2 N protein. Functional cellular T cell responses are suggestive of $CD4^+$:$CD8^+$ activity occurrence in these ratios 96%:54% in COVID-19 disease with further research required to clarify this. Compared to other respiratory viruses like influenza the SARS-CoV-2 S protein possesses higher mutational rates within the spike/ACE2 interface and emergent Omicron variants support this more recently denoted by BA1, BA2, BA2.75, BA4, BA5 and BQ1 [16]. Fortunately, many laboratory techniques now exist that facilitate accurate cell profiling through various techniques and allow comparisons of relevant immune cells. Therefore, in this paper B cells, neutrophils, Natural Killer cells (NK) and all T cell sub-types that contribute towards the overall response will be considered with mechanisms evolving around current research development. SARS-CoV-2 variations are evidenced since March 2020 to show S1 mutations above those of other respiratory pathogens with Omicron variants displaying resistance through evolutional mutations that are either prolonged in immunocompromised or by reinfections in healthy adults requiring clarity regarding all the other immunologically relevant cellular interactions of which T cells are arguably the most important in shaping development of all responses[13,14,17].

2. Methodology:

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This review cited electronic databases from September 1975 to October 2022 (e.g., PubMed and Google Scholar). Choosing articles was implemented using keywords to find relevant papers. "COVID-19", "B Cells", "T Cells", "NK Cells", "Innate", "Adaptive", "Cytokines", "Chemokines", "Adhesion Molecules", "Antibody", "Cluster of Differentiation", "Receptors", "Proteins", "SARS-CoV-2", "Serology", "Epithelial Cell", "Infection", "Immunology**".** Articles were chosen in accordance with topic relevance.

3. SARS-CoV-2 Infection

i) Respiratory Microenvironment

Respiratory tract organs affected include the nose, throat, larynx, trachea, bronchi, and lungs that are exposed to external antigens comprised of surface epithelial cell layers. An adult human lung surface area contains approximately 700 million alveoli, with surface area of 70 m^2 and diameter 200 -500μm covered by capillaries. Within this defined alveolar layer are ciliated type I pneumocytes, type II pneumocytes and alveolar Mϕ (AM) that regulate respiration, secretion of surfactant and immune cell regulation respectively alongside goblet cells, basal cells, and other cell types [18]. Early studies (n=7) in chronic SARS-CoV-2 disease show direct infection of type II pneumocytes through the glycocalyx and surfactant layer thereby compromising homeostatic barriers and valve functions through increased pressure of inhaled O_2 or exhaled CO_2 within nanobubbles across cell membranes where CO_2 is produced through the tricarboxylic acid (TCA) cycle [19]. The glycocalyx layer is known to contain an abundance of proteins that affect vascular function (e.g., syndecans) that can be degraded and affect the vasculature like metalloproteinases (MMP), heparanase, and hyaluronidase through the action of cytokines (IL-1β and others)[20,21]. This process of respiration is dependent on membrane thickness and gas solubility of O_2, N_2 and CO_2 nanobubbles [22] (see Figure 1). Type II pneumocytes are responsible for epithelial cell repair and renewal and are specialized secretory cells that produce surfactant consisting of 90% lipids (mainly saturated phospholipids) and 10% pulmonary surfactant proteins (SP) that contribute to the surface tensile maintenance utilizing dipalmitoylphosphatidylcholine (DPPC), and other SP, that are synthesized in the type II pneumocytes endoplasmic reticulum SP-A,SP-B, SP-C, SP-D. SP-B and SP-C are hydrophobic proteins with SP-A and SP-D possessing hydrophilic components intermixed that participate in pulmonary host defense. These act as valves with resulting dysregulation on clearance of a variety of pathogens. Specifically, SP-A and SP-D valve-like activities disrupted on pathogen clearance would result in dampened antigen presentation or allergy-induced immune function of cells [23].

ii) Case studies

Case studies clarify SP-D as a therapeutic target involved in increased dysregulation during chronic SARS-CoV-2 disease. Noteworthy publications contradict each other, and this may have been overlooked during the Influenza 2009 H1N1 pandemic. As numerous reports (n=10) clarify, there is extensive alveolar damage with endothelial injury, disruption of endothelial cell membranes, vascular thrombosis, occlusion of alveolar capillaries, oedema with angiogenetic vessel growth and lymphocyte migration [24]. The resulting mechanisms were documented as "cytokine storms" occurring between all leukocytes with resulting questions over neutrophils, monocytes and T cell function that utilize interleukins (IL), growth factors (GF), and chemokines (CXC) and respective receptors or ligands (e.g., CXCL4) that require further research [25]. SARS-CoV-2 pathogenesis therefore begins with disrupted epithelial cell membrane homeostasis with resulting syncytia formation and cell fusion resultant multinucleate cells [26–28]. This formation of syncytia could be initiated by transmembrane proteins (e.g., TMEM16) that regulate phospholipid rich cell membranes that include phosphatidylserine (PS) [29–31]. Braga et al utilized in situ hybridization (n=41) studies in deceased COVID-19 patients to clarify that SARS-CoV-2 infected fused cell syncytia contained napsin which process SP-B common to type II pneumocytes by screening 3000 compounds to find that niclosamide reduced calcium and its dependent calcium ion channel [29]. TMEM16 is a protein family known to be calcium dependent ion channels responsible for PS regulation in a normally calcium and arginine rich layer [29]. Concurrently it was discovered that SARS-CoV-2 ORF3a altered calcium regulating ion channel TMEM16F regulation by PS that can augment procoagulant activity through tenase and prothrombinase complexes, both key regulators of the coagulation pathway [30–32]. Therefore, such localized changes infer routes of SARS-CoV-2 entry within the epithelial micro-environment. Indeed, the carbohydrate rich glycocalyx layer covering mucosal epithelial cells also contains a mixture of mucin (MUC) glycoproteins, glycosaminoglycans, and other glycoproteins which extend and surround cilia and normally

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function to clear larger bacteria. Expansive research recently revealed that cilia, microvilli, and mucus function remain key for SARS-CoV-2 adhesion and receptor mediated entry into epithelial cells which appear to act as adhesives. MUC proteins are high molecular weight proteins that form mucus clusters. In COVID-19 disease initially (n=16) two types of mucins were extensively investigated, of which membrane-tethered MUC1 the gel-forming MUC5AC appeared at significantly elevated levels. Therefore, normal pathogenic clearance via mucin proteins could be disrupted, facilitating SARS-CoV-2 entry to allow viral persistence [33–36]. Importantly other researchers indicate in addition to ORF3a, other SAR-COV-2 proteins include E and ORF8a that can assemble to generate ion channels [37,38].

iii) Factors affecting SARS-CoV-2 induced COVID-19 fibrosis

COVID-19 disease can result in progressive fibrotic lung disease, one of the most concerning long-term sequalae where tissue stiffens with resultant decreased oxygenation and lung dysfunction. Post COVID-19 fibrosis (n=80) studies have identified this sequela in survivors, with lung architectural distortion and irreversible pulmonary impairment [39]. Pulmonary fibrosis can develop due to chronic inflammation and idiopathic, genetically driven, and age-related fibroproliferative processes. Pulmonary fibrosis is a renowned complication of acute respiratory distress syndrome (ARDS) [40]. Cytokine storm triggered by an aberrant immunological mechanism can initiate and promote lung fibrosis. For example, TGF-β was shown to be upregulated in COVID-19 disease and is a known cell differentiation factor, but pulmonary fibrosis can also be caused by fibroblast and myofibroblast accumulation and excessive collagen deposition [41]. Advanced age is an initial risk factor for the development of pulmonary fibrosis in COVID-19 disease [42]. Increased disease severity also includes comorbidities such as hypertension, diabetes, and coronary artery disease as a second risk factor [43]. The third risk factor is prolonged ICU stay and mechanical ventilation duration and alcohol abuse and smoking [44]. Pulmonary fibrosis is linked with blood leukocyte telomere length. Age-adjusted telomere length is a risk factor for post COVID-19 lung fibrosis. Short telomere lengths in blood leukocytes have been connected to the development of many subtypes of fibrotic interstitial lung disease, including idiopathic pulmonary fibrosis (IPF). Longer telomere lengths appear to be protective, suggesting that this genetic biomarker may be used to assess the balance of profibrotic and antifibrotic susceptibilities 4 months after COVID-19 disease, which is also associated with severity of illness and blood leukocyte telomere length [45]. Galectin-3, a carbohydrate-binding protein, is also produced in lungs by AM and epithelial cells. These cellular proteins have roles in COVID-19 infection progression, modulation, pulmonary associated inflammatory responses, and lung fibrosis. COVID-19-related lung fibrosis is associated with immediate widespread alveolar injury, oedema, hypoxia, and inflammation [46].

iv) Associated Proteins in SARS-CoV-2 infection induced COVID-19 disease

Coagulopathy and Cytokines

Studies indicate COVID-19-associated coagulopathy (CAC) is a causal factor in chronic COVID-19 disease with complexes formed between innate immune cells that affect coagulation and fibrinolytic processes through unknown mechanisms. Therefore, initial categorization of COVID-19 into vascular endothelial cell dysfunction, hyper-inflammatory response, and hypercoagulability document this aspect of SARS-CoV-2 induced pathology with resulting elevation in plasma levels of D-dimer, C-reactive protein, P-selectin, and fibrinogen [47]. Serum protein elevation is documented as a "cytokine storm" elevated or dysfunctional in SARS-CoV-2 induced chronic COVID-19 disease and many other pathologies [48]. That said it is indicated in comparison to influenza that these cytokines IL-1β, IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, TNF-α, and IFN-γ are more relevant to chronic COVID-19 disease progression. But also, transforming, and vascular endothelial cellular growth factors (TGF-β/VEGF)) along-side specific matrix metalloproteinases (MMP2, MMP3, MMP9) that represent tissue re-modelling proteins with specific chemotactic factors required to direct leukocyte chemotaxis between GC and throughout the body that include CXCL10 (IP-10), CCL2 (MCP-1), CCL3 (MIP1-α, and CCL11 [49–53]. However, in Middle East Respiratory Syndrome (MERS) IL-1β, IL-8, and IL-6 were highlighted whereas in SARS-CoV-2 CXCL10 does appear to be a key pleiotropic chemokine that has since been clarified in SARS-CoV-2 utilizing CXCR3 expressed on Mϕ, T cells, dendritic cells and both NK and B cells [54–56].

v) Role of Toll-Like Receptors (TLR) or TLR induced IFN dysregulation

In order to mount an anti-viral response, usually type I IFN is produced [57]. Current research contradicts this as type I IFN production presents as beneficial and detrimental in COVID-19

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disease; however, studies indicate that with MERS and indeed RSV that the timing of type I IFN production affects this [57,58]. Additional considerations are surface and cytosol pattern recognition receptors (PRRs) that initiate downstream signaling cascades utilizing NF-kB, type I IFN and inflammasome pathways. These include damage associated molecular proteins (DAMP) that encompass a myriad of proteins surrounding and within nuclear and extracellular space that include ten conserved Toll-like receptors (TLRs), retinoic acid-inducible gene-I-(RIG-I)-like receptors, Nod-like receptors (NLRs), AIM2-like receptors, and intracellular DNA and RNA sensors that can lead to production of pro-inflammatory or antiviral cytokines necessary for antigen specific adaptive responses [59,60]. For example, IL-1RA is a DAMP receptor that once released intracellularly binds to and initiates IL1α release and indeed case studies (n=71) did show this was the case in chronic COVID-19 disease concurrently with IL-10 which is largely immunosuppressive [61,62]. It is known that SARS-CoV-2 proteins are recognized by cellular sensors and therefore the role of TLR3/4/7 is of interest in terms of which immune cells express these. TLR3 is more abundant in NK cells, whereas TLR4 is more common in MF. TLRs transduce signals via MyD88 and TRIF. Most TLRs use MyD88 to trigger inflammatory cytokine production; TLR3 is the exception and signals exclusively through TRIF whilst TLR4 is unique in that it can bind and signal through either MyD88 or TRIF to nuclear transcription factors. Previous *in vitro* studies indicate that TLR3/7 may be associated with IL-1α, IL-1β, IL-4, and IL-6 release [63]. Therefore, other studies investigated the nature of TLR7 as a risk factor in severe COVID-19 disease [64]. The role of TLRs in immune cell signaling is largely unclear and will undoubtedly need further research but it is implicated in T cell signaling [65]. TLR4 is present on monocytes, MF, and dendritic cells (DC), and in some non-immune cells, like endothelial cells, and has a role in both LPS induced gram negative bacterial CD14 immune cell trafficking and interestingly may regulate RORγt$^+$ regulatory T-cell responses in colitis [66–68]. Indeed, new studies are clarifying the role of SP-A as we mentioned previously and it is entirely plausible that TLR4 expression has differential effects within different organ systems depending on activation; this was seen in neonates when TLR2/4 activation was shown to stimulate downstream extracellular-signal regulated kinase (ERK) and protein kinase B (AKT) with IL-6 pathways un-changed between children and adults [69]. Expression on both platelets and AM could affect thrombotic and immune pathways simultaneously with reduced expression on type II pneumocytes and confirmation in animal studies shown recently to link TLR4 to intestinal cytokine mRNA expression. [70–72]. TLR4 clearly has an influence on platelets though aggregation and P-selectin expression, and the formation of mixed aggregates between platelets and neutrophils and in microbes LPS triggers the synthesis and/or secretion of von Willebrand factor (VWF), platelet factor 4 (PF4/ CRCX4), and thromboxaneA2 (TXA2) alongside NETosis with CD11b upregulation and other adhesion molecules (supplementary data sheet 2) [[73,74].

Originally identified in 1957 by Isaacs and Lindemann, IFNs were found to be in secretions and could both inhibit viral growth and tumour growth. They are currently classified into three groups: Type I, II, and III. Type I IFNs consist of IFN-α, IFN-β (also IFN-δ, IFN-ε, IFN-κ, IFN-τ, IFN-ω, and IFN-ζ) but also within Type II is IFN-γ, whilst type III IFNs encompass IFN-λ [75] Noteworthy, studies indicate that with MERS and RSV that timing of Type I IFN production affects this [57,58]. With regards to SARS-CoV-2 sensitivity to IFN, early case studies indicate SARS-CoV-2 sensitivity to IFN-α and IFN-β *in vitro*, however more recent tissue studies indicate that IFN-α, IFN-β response may paradoxically facilitate the propagation of COVID-19 from the respiratory epithelium to the vasculature through direct endothelial cell infection [76,77]. Recently a type III IFN-λ has been investigated and is under clinical research following earlier studies (n=257) that document reduced IFN-λ2 during chronic COVID-19 disease [78]. The cellular source of SARS-CoV-2 infection IFN is largely unknown presently, as IFN receptors are located within B cells, monocytes, MF, T-cells lymphocyte, glial cells, neurons, plasmacytoid dendritic cells and others. Interestingly epithelial response *in vitro* studies show that IFN-γ can promote SARS-CoV-2 infection in cell culture and enhance cell differentiation within enterocytes in vitro [79]. Type III IFN, IFN-λ, only discovered in 2003, has been well documented so far as activated by bacteria including IFN-λ, Salmonella, Listeria, Staphylococcus aureus, Mycobacterium tuberculosis and others [80]. It is of note that type II IFN and type III IFN can be secreted by NK and T cells and few studies document whether type III IFN affects antibody class-switching. Although epitope determinants of SARS-CoV-2-specific CD4$^+$ T cell lines reveal SARS-CoV-2 M protein-driven dysregulation of IFN gene signature (ISG) like chronic COVID disease (ISG15, IFITM1, IFI16, MX1, STAT1, OAS1, IFI35, IFIT3 and IRF7) [81].

4. Factors in Antibody Production in B cell Development

i) B Cell Development

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B lymphocytes represent 10% of white blood cells (leukocytes). These are central to innate immune responses as pathogen sensors, throughout their development in germinal centers (GC) and distribution throughout the lymphatic system network by secretion of immunoglobulins (Ig) determining detection and neutralization of antigens through cellular development processes [82]. B cells respond to non-host antigens dependent on receptor that include antibodies shed from the cell surface (e.g., IgM, CD79a and CD79b) (see Figure 1).

B cell development from hematopoietic precursor cells occurs in stages from pro-B cells, pre-B cells, immature B cells, and growing into mature B cells in fetal liver and bone marrow. B cell responses are defined by CD markers evolving into mature B cell subpopulations, such as B-1, B-2, and regulatory B cells [83,84]. This field has evolved in 2020 and beyond to those of novel B cell subsets defined by phenotypic CD markers with single cell sequencing allowing greater depth of characterization. Remarkably, B lymphocytes synthesize up to 10^{11} antibodies, or B cell receptors (BCR), within a host. BCRs undergo clonal selection and somatic hypermutation (SHM) leading to specificity of antigenic epitope protein recognition. BCR consists of a transmembrane section and extends intracellularly through cytoplasm with protein sequences that depend on co-activation or stimulation from other proteins to activate B lymphocytes. Other CD molecules define B cells lineage (e.g., CD19, CD21). These are relevant to cell residing locations, developmental stages, maturation, and activation state. CD10 expression occurs on first-stage B cell lineage cells (e.g., pro-B, pre-B cell, and GC) and can be downregulated throughout maturation [85] with others shown on figure 2. Also, CD27 exclusively resides within memory B plasma cells whilst CD5 characterizes B-1 cells. All B lymphocytes utilize T cell interactions (TCR) to stimulate proliferation and differentiation, resulting in pre-GC memory B cells (pre-GC MBCs) and short-lived plasma cells (SLPCs) that produce low affinity early antibodies. Other B cells reach the germinal center (GC) where antibody affinity and selection can occur by clonal selection/SHM modifying structure via class-switching recombination (CSR), resulting in long-lived plasma cells (LLPCs) and memory B cells (MBCs) with specific antibody isotypes but also plasmablasts that produce immunoglobulins (Ig) of 5 main isotypes that occur as multimeric proteins (IgM, IgG, IgA, IgE and IgD) in normal host specific immune responses indicated within these ranges IgG: 80%, IgA:15%, IgM:5%, IgD:0.2% with trace amounts of IgE (see table 1) [86].

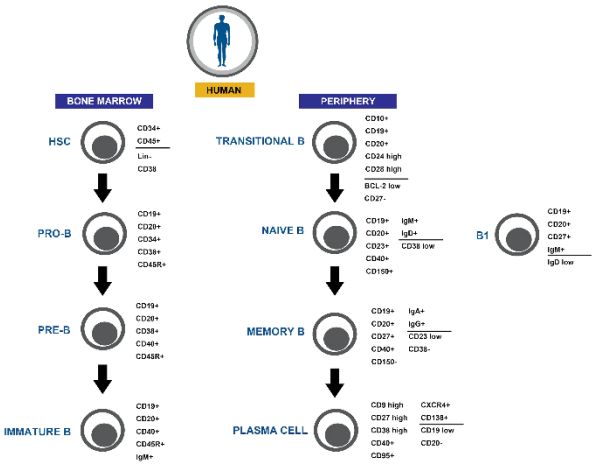

Figure 2: B Cell Development

ii) Antibody Isotypes and Class-Switching

An antibody (Ig) is a structural monomer unit of β-strands and complementary determining regions (CDRs), composed of two light chains and two heavy chains associating with each other through disulphide linkages secreted by B cells in response. However, a joining chain (J) chain is also present within IgA and IgM classes allowing the formation of dimer or pentamer structures, which contrasts with the other monomeric antibody isotypes. Light chain classes have a constant and a variable domain and heavy chains possess five isotypes with individual roles associated in the immune system; IgG, IgD, IgA (possess 1 variable and 3 constant chains) with IgE and IgM (1 variable and 4 constant chains). The predominant immunoglobulin IgG (80%) is a tetrameric 150kDa quaternary structure globular protein with IgG occurring as four conserved sub-types (IgG1, IgG2, IgG3, IgG4) and varying effector functions [87]. Ig structures that encompass the BCR

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contain antigen binding F(ab) or constant F(c) structures referring to the top polypeptide receptor or tail polypeptide structures spanning the phospholipid bilayer and changes to either polypeptide conformation could affect either any of the SARS-CoV-2 / antibody transduction that effectively tethers and effects downstream function of antibody molecules either inside or outside the cytoplasmic membrane in secretions or in sera. In short, an array of associated functions can result as individual isotypes known by Greek terminology have variable antibody isotypic receptors (e.g., FcγR denotes antibody isotype IgG receptor), resulting in an array pf effector function that can include phagocytosis, antibody-dependent cell-mediated cytotoxicity (ADCC), or complement fixation through other FcγR receptors (CD16, CD32, and CD64 and sub-types) amongst others [88,89]. IgG dominates during natural infection and vaccine responses in sera.

Isotype	Serum Level (mg/mL)	Molecular Weight (monomer)	Complement Activation -	Half Life
IgA1	0.6–3	160 (monomer)	-	5.5
IgA2	0.06–0.6	160	-	5.5
IgM	1.5	970	+++	5-10
IgE	5×10^{-5}	188	-	2
IgG1	3.8–11.4	146	++	23
IgG2	1.5–6.9	146	+	23
IgG3	0.2–1.7	165	+++	7
IgG4	0.08–1.4	146	-	23

Tabel 1: Antibody Isotypes Concentrations in Sera and Complement Activation Ability

IgA (monomer,) 160kDa, represents 15% of total Ig and co-exists in dimeric secretory form, with monomeric subunits adjoined by a 15kDa J chain (335kDa) in the upper respiratory tract. IgA1 and IgA2 is secreted by B cells in infection usually in ratio 90% IgA1 to 10% IgA2[90]. IgA is found in secretions including tears, saliva, sweat, colostrum, genitourinary tract , prostate and respiratory epithelium and in trace amounts in blood. IgA molecules are made up of two identical heavy chains and two identical light chains. Secretory IgA composition occurs approximately in ratios within colonic secretions IgA1-90%: IgA2-60% [91]. IgA production occurs from plasma B cells that are actively transported to mucosal surfaces effecting activity via respective FcαRI (CD89) receptors present on neutrophils, basophils, eosinophils, monocytes, Mϕ, and dendritic cells, known as a key regulator modulating host protection from bacterial infection and sepsis. A key receptor CD64 was characterized around 2015 as a biomarker that can occur within the gastrointestinal tract, prostate and respiratory epithelium and in trace amounts in blood [92–94]. IgA is key in mucous membrane pathogen clearance (e.g., H. Influenza) and ingested allergens such as peanuts. Whereas IgM to IgE (monomer) class switching is necessary for regulation of allergy responses during hypersensitivity immune responses. Patients can experience anaphylaxis where high IgE levels and other mast cells and mediators (e.g., histamine) result in vasodilatory complications with prescribed treatments available to reduce IgE mediated anaphylaxis. IgM to IgD (monomer) class switching and IgD function remains unclear in the literature, but researchers have theorized that this immunoglobulin is important in B cell maturation in transition from autoreactive to antigen specific responses thus playing a key role in clinical morbidity and mortality [95]. As mentioned, IFN could play a key role in B cell antibody switching. The duality of Type 1 / II IFN was shown to exist, before type III IFN was discovered in 2003; for example, IFN-α inhibition of an IgE binding factor (BF) produced by IFN-γ-stimulated monocytes effecting downregulating respective high affinity FcεR expression on B Cells. But also, IL-4 was indirectly found to affect soluble CD23 expression and IgE production [96]. IgE and IFN-α can reduce TLR-9 receptor expression and TLR-7 signaling to disrupt IFN production. This has been heavily documented with antihistamine therapeutics. Moreover, anti-IgE drugs such as omalizumab reduce the severity and duration of COVID-19. In addition to its anti-IgE effect, omalizumab can inhibit some of the inflammatory functions of neutrophils which was seen with IgE in RSV pathology occurring presumably by blocking FcεR [97].

B cells utilize MHC Class II receptor signaling complexes, found on antigen-presenting cells (APCs) like dendritic cells (DC), monocytes and Mϕ, endothelial cells, epithelial cells, and B cells. This MHC class II complex facilitates extracellular peptides that are endocytosed, digested within lysosomes, by presentation to the cell surface effector cells. MHC Class II complex is encoded by human leukocyte allele (HLA) genes divided into sub-types corresponding to HLA-DP (also HLA-DM, HLA-DQ, HLA-DR, and others). These are highly polymorphic and vary depending on genetic factors, thus the variable HLA gene complex affects presentation of SARS-CoV-2 antigens to effector cells with cytokine stimulation and antibody secretion. The BCR protein complex non-

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covalently binds to other proteins and membrane-bound Ig receptor (mIg), Ig-alpha (CD79a) and Ig-beta (CD79b). This CD79a/CD79b complex transduces signals giving linkage to mIg heterodimeric disulphide complexes that contain immune tyrosine-based activation motif (ITAM) sequences vital for B and T cell signal transduction. ITAM sequences transmit activation signals from BCRs, through tyrosine to the cell cytoplasm. Tyrosine phosphorylation by protein kinase cytoplasmic cellular signaling occurs with proteins. The inducible co-stimulator (ICOS) homodimeric protein promotes T/B cell signaling through CD40 and B7/CD40L. Such co-stimulatory interactions are vital for progressive adaptive immunity development and lymphocyte activation. Inefficient adaptive immunity can cause significant impairments in immunoglobulin isotype switching. Moreover, ICOS ligand B cell interaction with activated T cells is essential for the formation of GCs, and optimal production and sustained release of other serum / sera antibodies (IgA, IgE, and IgG sub-types) to maintain effective host immunity. However, such HLA genetic variations can be associated with differential risk factors in many disorders.

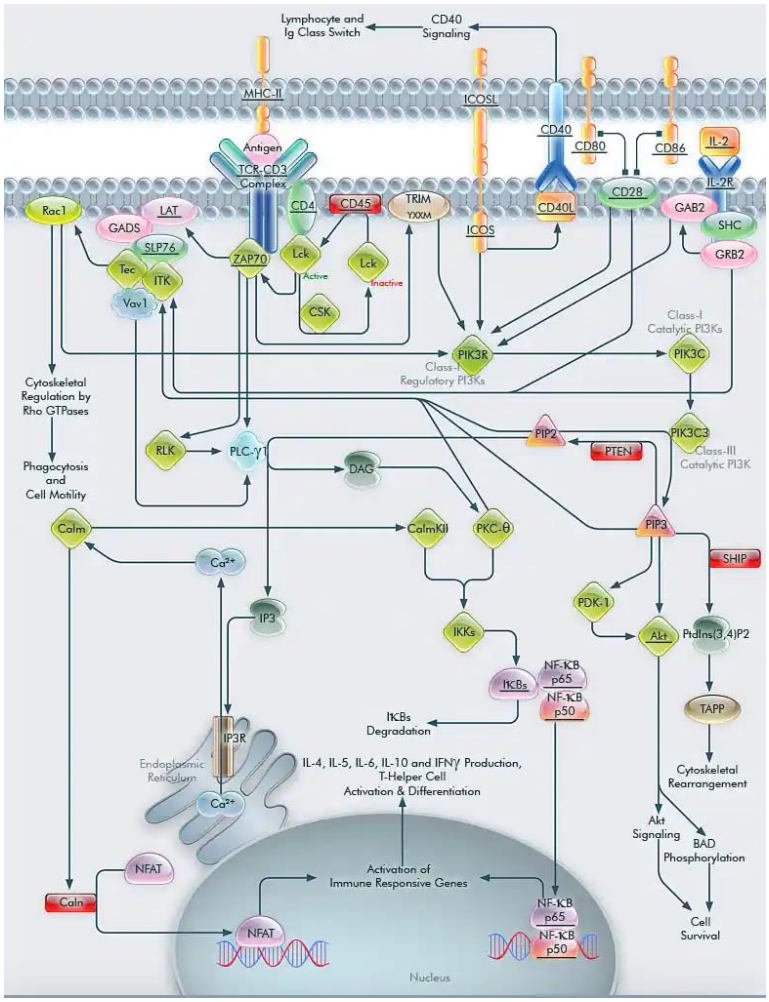

Figure 3: ICOS-ICOSL pathway in T-helper cells

iii) Role of B Cell Markers in Current Research

CD19 has long been used as a B cell biomarker [98]. Other pathologies are characterized by another marker namely CD64 within neutrophils for example in sepsis [94]. In recent years this has expanded to encompass functionality of B cells by receptors expressed at different maturation stages of naive B cells, unswitched memory B cells, switched memory B cells, and double-negative (DN) B cells. Some studies suggest there is no consensus on DN B cells, however these DN B cells have been clarified (See Table 3). Further DN B cell analysis of CD11c has refined these into subsets that express CXCR5 that are hypothesized to emerge from naive B cell activation outside of the GC [99]. Researchers expanded this recently to two other sub-types, DN3 and DN4 (See Table 2). Indeed, it can now be seen that there could be a putative role for unknown B cell sub-types such as DN2

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downregulating CXCR5 or other direction specific receptors like CD62L (L selectin) that are usually expressed (Supplementary data sheet) [100]. Enrichment within certain DN B cell subsets has been discussed to play a key role in other comparatively well characterized autoimmune diseases [multiple sclerosis (MS), systemic lupus erythematosus (SLE), myasthenia gravis (MG), rheumatoid arthritis (RA)] [99,101,102]. For example, $CD27^{+}IgD^{+}$ has been suggested to have impaired gene signaling in RA through VH3-23D to VH1-8 affecting production or rather reduced BCR diversity during selection [103]. Thus, recent sub-types have examined the DN CD11c phenotype (n=18) to show these in autoimmune pathologies compared to healthy controls (SLE, Sjogren's syndrome). Furthermore, B cells (CD19) expressing CD11c+ together with elevated levels of CD69, Ki-67, CD45RO, CD45RA, as metabolic markers and B cell memory phenotype markers respectively along with lack of DN cell markers CD21 could well escape normal immune cell regulation [104,105]. Therefore, depletion of B cell subsets has also been examined in age referred to as age-associated B cells (ABCs) affecting production of autoantibodies [106,107].

B Cells	Markers			Chemokines	Ig	Amount	
Naïve B Cells	$CD27^{+}$				IgD^{+}	29-69	[101,108]
Unswitched B Cells	$CD27^{+}$	-			IgD^{+}	5-22	[109]
Switched B cells	$CD27^{+}$				IgD^{-}	8-43	[109][109]
B cell DN1	$CD27^{-}$	$CD21^{+}$	$CD11c^{-}$	$CXCR5^{+}$	IgD^{-}	4-6	[102,110]
B Cell DN2	**$CD27^{+}$**	**$CD21^{-}$**	**$CD11c^{+}$**	**$CXCR5^{-}$**	**IgD^{-}**		[111,112]
B Cell DN3	**$CD27^{-}$**	**$CD21^{-}$**	**$CD11c^{-}$**		**IgD^{-}**		[113]
B Cell DN4	$CD27^{-}$				IgD^{-}		[113]

Tabel 2 Adapted from Li et al [114]

Currently B cell memory and production of S protein specific Ig was initially measured at six months by production and expression of IgG with other cellular markers being researched as to changes occurred and it can now be seen that a putative role for unknown B cell sub-types such as DN2 downregulating CXCR5 or other direction specific receptors like CD62L (L selectin) that are usually expressed [100]. Enrichment within certain DN B cells subsets has been discussed to play a key role in other comparatively well characterized autoimmune diseases [multiple sclerosis (MS), systemic lupus erythematosus (SLE), myasthenia gravis (MG), rheumatoid arthritis (RA)] [99,101,102]. For example, $CD27^{+}IgD^{+}$ has been suggested to have impaired gene signaling in RA through VH3-23D to VH1-8 affecting production or rather reduced BCR diversity during selection [103]. $CD19^{+}$ $CD24^{+}$ $CD27^{+}$ $CD38^{+}$ indicate responses to SARS-CoV-2 S protein specific B cells increasing to 36%, however limited data currently exists, although further details can be seen regarding the two other relevant transitional B cells (Supplementary Data Sheet) [115]. Earlier in 2022 Plume et al carried out a unique study (see Table 2) examining the antibody main isotypes against all SARS-CoV-2 proteins and most fragments, and they indicated that seroconversion at day 20 may cause issues as 97.3% reacted against the chosen epitopes of SARS-CoV-2 but E protein was not looked at in this study. Interestingly in contrast with other studies it was found that a 32 amino acid peptide (V551-L582) in the currently mapped RBD domain could be an immunodominant B Cell epitope equating to 58.7% of IgG samples tested [116][101] (See table 2). As above, naïve B cells expressing $IgD^{+}CD19^{+}CD27^{-}$ (see table 3) can be sole predictors of antibody titrations compared to control groups with high significance (P=0.009)[117]. Nonetheless, chronic COVID-19 disease patients presenting with DN (IgD^{-} $CD27^{-}$) B cells are shown to experience worse disease severity and complications. The DN1 subset is noteworthy holding potential for early activated memory cells whereas the DN2 cells encompass antibody plasmablast (PB) secreting cells that have been primed beforehand, however it remains uncertain the impact that each DN B subtype and mechanistic properties has on disease resolution [118].

iv) IgG / IgA Infection Antibody Responses SARS-CoV-2

Responses in SARS-CoV2 Infection

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IgG1 and IgG3 were initially associated with severe disease (n=123) in COVID-19 disease in older age groups with accompanying irregularities in neutralizing antibodies, chemokines and T cell responses which is an anomaly, as IgG3 was previously thought to provide enhanced pathogen responses [119,120]. However, IgG deficiency has been observed to be associated with increased mortality risk in Chronic Obstructive Pulmonary Disease (COPD) patients (n=489) in these ratios 56% IgG1: 27%: IgG2: 24% IgG3: 31% IgG4 [121]. A previous study (n=105) comparing serology of hCoV-229E, hCoV-OC43, hCoV-NL63 and hCoV-HKU1 elucidated that participants show an antibody response with respective IgG responses as 99%:100%:98%: 91% with IgA in nasal wash samples detected between 8% to 31% of participants [122]. Lower pathogenic hCoVs represent 15–30% of common cold respiratory tract infections in humans each year with seropositivity estimated at 90% in adult indicating that T role requires further clarification [123]. An effective non-coronavirus antigen comparison would therefore be influenza haemagglutinin (HA) and neuraminidase (NA) proteins which occur seasonally. In these cases, research indicates an increase in serum IgM specific HA antibodies during the 2009 H1N1 pandemic in these ratios: IgM (86-94%), IgG (100%), and IgA (76 to 96%) [124,125]. Therefore SARS-CoV-2 is novel by not eliciting a higher level of secretory IgA like lower pathogenic hCoVs. In fact, SARS-CoV-2 induced chronic COVID-19 disease did stimulate elevated levels of five serum antibody types of IgM, IgG1, IgA1, IgG2, and IgG3 at day 3 with IgA1 seemingly temporal at day 7 [126–128]. It was therefore demonstrated in chronic COVID-19 disease that significantly higher IgG1, IgG2, IgG3 occurred at day 3 alongside transient elevation in IgA1 disappearing by day 7 to be crucial but also mature neutrophils expressed significantly increased levels during maturation of CD32 (FcγRIIa), CD16b (FcγRIIIb), and CD89 (FcαR), the main receptors. Patients that possess dominant IgA responses were found to have greater risk of mortality in severe COVID-19 diseases experiencing dysregulated myelopoietic responses. High IgA to low IgG titrations cause pathological consequences in the host involving decreased pathogenic phagocytosis, increased cellular apoptosis, and increased NETosis as reported in late stage fatal COVID-19 cases. Patients possessing a high IgG to IgA ratio experience greater inflammatory dampening, and immune responses, resulting in better prognoses and early-late-stage disease resolution [117]. Limited data exists to reveal why SARS-CoV-2 induced COVID-19 disease exhibits such a novel antibody profile regarding IgG1/IgA1 responses. It is considered that alterations to the Ig structure can produce complications either increasing infections or immune complex formation and in other pathological diseases like dengue antibody-dependent enhancement (ADE) this did occur and was caused by IgG antibodies. These changes include glycosylation (glycan or carbohydrate adjoining hydroxyl or other functional groups) but also fucosylation (transfer of a sugar fucose from a GDP-fucose to other proteins or glycans) and therefore this could affect leukocyte extravasation and selectin mediated binding through cellular membranes which is recognized as a potential factor in cancer therapeutics [129,130]. Therefore, studies that occurred during the pandemic (n=33) examined this in chronic COVID-19 disease to confirm that IgG against the SARS-CoV-2 RBD protein could potentially initiate macrophage release of IL-1β, IL-6, IL-8, TNF [131]. However, IgG3 and IgM are inferred to be responsible for 80% of neutralization of SARS-CoV-2 with suggestions that IgG3 glycosylation affects SARS-CoV-2 binding specificity to the S protein [128,132]. As mentioned earlier glycosylation can occur where an N-linked glycan forms within the IgG-Fc region. In accordance with examining the IgG sub-types a recent study from Brazil examined the avidity of IgG (n=47) to SARS-CoV-2 proteins to show an increase of IgG1 and IgG3 levels at day 8, and IgG4 levels less detectable during the study period. Mortality at 8–21 days showed higher anti-RBD IgG4 levels in comparison with the recovered which contradicts other studies but is relatively unknown with regards to IgG4 pathology research [133]. Early in 2020 initial screens of N/S/E SARS-CoV2 proteins in smaller cohorts (n=320) did indicate that anti-N IgG and anti-N IgA produced in response to SARS-CoV-2 was generated and also IgG antibodies were produced to S1 protein and the E protein, but also that anti-E protein antibodies were not significantly higher which is indicative of the current immunogens within clinical trials and those used in lateral flow testing [134].

Antigen	Mild			Moderate			Severe			Total		
	IgG	IgA	IgE	IgG	IgA	IgA	IgG	IgA	IgE	IgG	IgA	IgE
Spike (FP)	94.7	16.7	66.7	100	38.5	92.3	100	54.5	90	97.3	30.1	79.7

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Stable Spike Trimer	97.4	25	12.1	100	42.3	50	100	54.5	60	98.7	35.6	33.3
RBD	92.1	22.2	0	100	42.3	3.85	100	63.6	10	96	35.6	2.9
S1sub	89.5	22.2	0	100	42.3	34.6	100	63.6	20	94.7	35.6	15.9
S2sub	100.0	25	3.03	100	42.3	19.2	100	54.5	20	100	35.6	11.6
N_{fl}	94.7	19.4	66.7	100	46.2	96.2	100	45.5	100	97.3	32.9	82.6
M_{fl}	84.2	13.9	0	100	30.8	23.1	100	45.5	0	92	24.7	8.7
Protein Fragments and Predicted Epitopes												
S1	5.26	0	0	7.69	0	0	27.3	0	0	9.33	0	0
S2	31.6	0	0	26.9	0	0	72.7	0	0	36	0	0
S3	26.3	0	0	26.9	0	0	54.5	0	0	30.7	0	0
S4	5.26	0	0	15.4	0	0	36.4	0	0	13.3	0	0
S5	34.2	0	0	23.1	0	0	36.4	0	0	30.7	0	0
S6	13.2	0	0	15.4	0	3.85	0	0	0	12	0	1.45
S7	42.1	2.78	15.2	69.2	0	34.6	90.9	0	30	58.7	1.37	24.6
S8	13.2	0	0	15.4	3.85	0	18.2	0	0	14.7	1.37	0
S9	28.9	0	0	26.9	0	0	45.5	0	0	30.7	0	0
S10	15.8	0	0	15.4	0	0	18.2	0	0	16	0	0
S11	0	0	0	7.69	0	7.69	18.2	0	0	5.33	0	2.9
M1	18.4	0	0	38.5	0	0	54.5	0	0	30.7	0	0
N1	28.9	0	0	30.8	3.85	0	54.5	0	0	33.3	3.85	0
N2	42.1	0	0	53.8	0	0	63.6	0	0	49.3	0	0
NSP1	36.8	0	0	15.4	0	0	36.4	0	0	29.3	0	0
NSP2	7.89	0	0	11.5	0	0	27.3	0	0	12	0	0
NSP3	2.63	0	ND	11.5	0	ND	9.09	0	ND	6.67	0	ND
NSP4	2.63	0	ND	3.85	0	ND	0	0	ND	2.67	0	ND
NSP5	31.6	0	ND	34.6	0	ND	45.5	0	ND	34.7	0	ND
ORF3b	2.63	0	ND	3.85	0	ND	18.2	0	ND	5.33	0	ND
ORF8	7.89	0	ND	7.69	0	ND	18.2	0	ND	9.33	0	ND
ORF8_1	0	0	ND	0	0	ND	0	0	ND	0	0	ND
ORF_2	5.26	0	ND	15.4	0	ND	27.3	0	ND	12	0	ND

Tabel 3 Overall Serological Response Quantification in SARS-CoV-2 Infection (%) [116]

v) IgG / IgA Infection Antibody Responses In other pathologies

In Ebola virus (EBOV) survivors of the 2013 – 2016 outbreak the roles of IgG1/ IgA1 and IgA2 indicated a trend of polyfunctional IgG1 being more immunologically beneficial against each of the four EBOV proteins GP, secreted GP (sGP), nucleoprotein (NP), and matrix protein VP40 which offers biologic plausibility [135]. Dengue Fever Serology IgA / IgG

Role of B Cell Markers of SARS-CoV-2 Infection

Antibody responses to SARS-CoV-2 mRNA induced vaccination can be attained in patients on anti-CD20 therapy via the onset of B cell repopulation [136]. In the absence of B cells, a strong T cell response is generated which may help to protect against chronic COVID-19 in this high-risk population [136]. Therefore, it is essential to understand the nature of this response. Researchers in 2020 found chronic COVID-19 disease (n=52) DN1 B cells decrease with increases in DN2 B cells but significant increases in chronic patient severity with DN3 cells.

Other B cell subsets were therefore investigated to discover an undefined subset of B cells named "transitional B cells or Tr" that correlate with improved clinical outcome as measured by B cells expressing more CD24 than CD21 [118]. This was an interesting finding because CD24

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expression is known to affect cell migration, invasion, and proliferation whilst expression or lack of CD21 is associated with B cell memory and complement proteins. CD21 is also expressed on follicular dendritic cells and is known to associate as a complex with complement proteins (C3dg, C3d, and inactive C3b) on the antigen surface, together with CD19/CD81 [110,118,137]. Interestingly increased Tr cells did correlate with COVID-19 disease routinely used blood protein markers detected including neutrophil/lymphocyte ratio, acute phase proteins, ferritin levels, D-dimer, and others. The exact nature of DN B cells requires further clarification, as subsets are associated with SLE [138]. As before the B Cell DN1 reduction / DN3 increase in chronic COVID-19 was accompanied by high levels of CD69 and CD89 in DN2 cells alongside what appears to be DN2 selection of IgG but also suggests that DN3 cells do produce VH4-34 IgG autoreactive antibodies [113]. There were initial indications that germline Ig variable heavy chain VH4-34 showed decreased SHM frequencies which would affect B cell Ig maturation through SHM process [139]. Unswitched memory B cells ($CD27^+$ IgD^+) historically are part of normal and pathological immune responses with reduced overall IgM secreting B cells for example in RA thought to occur due to gene recombination contributing to antibody selection by VH3-23D to VH1-8 [103]. Interestingly, the BCR repertoire of these cells was altered in RA, exhibiting some of the same markers as DN2 cells, such as CD11c and FcRL5, and transcription factor (T-bet) [140,141]. While antibodies generated by B cells are historically well characterized it is unclear why SARS-CoV-2 generates high antibody responses in chronic severity and not in acute. The timing of an antibody response is important in antibody-based therapeutics, as drug application influences patient outcomes [142,143]. Naïve B cells are activated with the assistance of follicular T (T_{FH}) cells [144]. Therefore, this novel antibody expression caused by SARS-CoV-2 infection induced chronic COVID-19 disease found to be of two classes of IgM, IgG1, IgA1, IgG2, and IgG3 requires analysis. Recent analysis of vaccine immunogens (e.g., Pfizer/BioNTech BNT162b2) indicate Ig expression by spike-specific B cells at six months after 2/3 doses in these ratios IgG:77.46%, 61.33% with concurrent IgA: 7.37%, 3.04% and IgM:12.30%, 24.97 to note significant reduction in IgG / IgA with significant increase in B cell specific IgM at six months after third dose compared to three weeks after first dose [145]. As discussed earlier, B Cells develop in GCs and through a small cohort (n=15) study a role for circulating T_{FH} cells was elucidated to show S protein specific B Cells develop and undergo SHM but at five months 66% of this cohort had B memory cells to vaccine immunogens and this research was suggestive that there was slight increase in nAb [146]. Concurrent with other studies unsurprisingly minor differences in memory-switched cells as the main population represented (median: 59.92%) but also their analysis examined SARS-CoV-2-specific B cells markers CD27 and CD38 markers, to show a significant increase in $CD27^{high}CD38^{high}$ plasmablasts (PB) in recovered compared to uninfected subjects at six months and IgD^+CD27^+ and IgD^-CD27^+ B cells were significantly reduced in chronic SARS-CoV-2 infection [147]. B cells control antibody secretion and reports indicate that IL-10 and IL-21 are responsible for B cell class-switching to IgG1, IFN-γ class-switching to IgG2 and TGF-β switching from IgA1 to IgA2 responses [126]. Research shows that IgG1 and IgG3 (n=123) do correlate in chronic SARS-CoV-2 severity with cytokine IL-1β response [119]. IgG2 is thought to be more relevant to bacterial response to capsular polysaccharide antigens [120]. Concurrent in vitro studies also indicate that SARS-CoV-2 IgA1 and IgG3 may have a protective neutralizing effect in SARS-CoV-2 infection. [148,149]. Further research would be required to clarify this. Other studies (n=82) confirm that in chronic SARS-CoV-2 infection within seven days serum antibody response is 60%: IgA, 53.3%: IgM 53.3% and IgG:46.7% with IgG reaching 100% by day 21 [150][156][151]. Polymorphonuclear neutrophils (PMN) are granular and trilobed, being the most common circulating leukocyte, representing between 40%-80% of leukocytes in normal adults. Neutrophil infiltration in respiratory tissues is characteristic of many inflammatory diseases [152]. Neutrophils are granular acting against antigens by dispersing azurophilic cytoplasmic granules using the actions of proteolytic enzymes (e.g., myeloperoxidase, elastases, proteinase 3) but also lactotransferrin, lysozyme or reactive oxygen species (ROS) which are also anti-microbial for clearing pathogens [153,154]. Pathogenic stimuli trigger cellular calcium release via endoplasmic reticulum (ER), resulting in activation of protein kinase C (PKC) and assembly of the NADPH oxidase complex generating ROS. Neutrophils form from hematopoietic stem cells in marrow and are short-lived between 1-7 days and traverse cell membranes by selectin-dependent capture and integrin mediated adhesion (Supplementary Table 2/3) after which migration to tissues occurs and survival for 1 -2 days whilst circulating and clearance by macrophages through the process of phagocytosis. Development of neutrophils occurs in bone marrow from progenitor neutrophils and can be broadly classified according to CD markers as $CD81^+CD43^+CD15^+CD63^+CD66b^+$ that differentiate into immature neutrophils expressing $CD11b^+CD66b^+CD101^{+/-}CD10^-CD16^{+/-}$ before maturing in the bone marrow to express $CD11b^+CD66b^+CD101^+CD10^+CD16^+$[155]. CD16 is co-expressed on other cells including NK cells, monocytes, Mϕ, and certain T cells [155]. CD16 is known as FcγRIII with sub-types including CD16a and CD16b (FcγRIIIa / FcγRIIIb), whilst CD11

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and specifically CD11b are thought to be more relevant to migration and lung inflammation [156–158]

ii) Neutrophils in SARS-CoV2 Infection

Polymorphonuclear neutrophils (PMN) are granular and trilobed, being the most common circulating leukocyte, representing between 40%-80% of leukocytes in normal adults. Neutrophil infiltration in respiratory tissues is characteristic of many inflammatory diseases [152]. Neutrophils are granular acting against antigens by dispersing azurophilic cytoplasmic granules using the actions of proteolytic enzymes (e.g., myeloperoxidase, elastases, proteinase 3) but also lactotransferrin, lysozyme or reactive oxygen species (ROS) which are also anti-microbial for clearing pathogens [153,154]. Pathogenic stimuli trigger cellular calcium release via endoplasmic reticulum (ER), resulting in activation of protein kinase C (PKC) and assembly of the NADPH oxidase complex generating ROS. Neutrophils form from hematopoietic stem cells in marrow and are short-lived between 1-7 days and traverse cell membranes by selectin-dependent capture and integrin mediated adhesion (Supplementary Table 2/3) after which migration to tissues occurs and survival for 1 -2 days whilst circulating and clearance by macrophages through the process of phagocytosis. Development of neutrophils occurs in bone marrow from progenitor neutrophils and can be broadly classified according to CD markers as $CD81^{+}CD43^{+}CD15^{+}CD63^{+}CD66b^{+}$ that differentiate into immature neutrophils expressing $CD11b^{+}CD66b^{+}CD101^{+/-}CD10^{-}CD16^{+/-}$ before maturing in the bone marrow to express $CD11b^{+}CD66b^{+}CD101^{+}CD10^{+}CD16^{+}$[155]. CD16 is co-expressed on other cells including NK cells, monocytes, Mϕ, and certain T cells [155]. CD16 is known as FcγRIII with sub-types including CD16a and CD16b (FcγRIIIa / FcγRIIIb), whilst CD11 and specifically CD11b are thought to be more relevant to migration and lung inflammation [156–158]. Neutrophils are distinguished by a p**olymorphonuclear appearance** (PMNs) that migrate between vasculature using several receptors CD11c, CD13, CD15, CD16, CD33, CD62L, CD63, CD66, CD88, CD141, defensins, lysozyme and MPO.

iii) Neutrophils in SARS-CoV2 Infection

During chronic COVID-19 disease it is believed that neutrophils form neutrophil extracellular traps (NETs), in which parts of the nucleus together with granules actively released via so termed "NETosis" or neutrophil apoptosis [159]. The exact mechanisms of NETosis contribution remain unknown [160]. Therefore, case studies (n=64) focused on identifying neutrophil subsets in disease recently to show some appear to suppress stimulation of IFN-γ production with unknown subsets that stimulate T cell proliferation but fail to activate T cells [161]. Several authors suggest that host driven immune response lacks production of type I and III interferons in conjunction with elevated chemokines with IL-6 a causal factor in coronavirus pathology [162,163]. Therefore, whilst IL-6 could be the predominant cytokine regulator of NETosis other protein markers are now elucidated being extracellular DNA (ecDNA), neutrophil elastase (NE) activity or myeloperoxidase-DNA (MPO-DNA), and these correlate with disease severity measured in neutrophils by markers $CD33^{low}CD16^{+}CD11b^{+}$ [164]. Researchers recently found (n=155) that elastase, histone-DNA, MPO-DNA, and free double stranded DNA (dsDNA) were increased with a concurrent DNase reduction and exacerbation of neutrophil stimulation occurring via IL-8, CXCR2 and DAMPs with impaired degradation of NETs via DNase 1 and DNase 11L3 which are suggested to act as regulators of neutrophil DNA metabolism [165]. However, a comprehensive neutrophil analysis (n=384) utilizing non-negative matrix factorization (NMF) classified 6 individual cell states defined by inflammatory signature (ISG) to indicate that concordant IgA1:IgG1 ratios are elevated in coronavirus disease mortality with IgG indicating antibody-dependent neutrophil phagocytosis with IgA2 inducing apoptosis [166]. No studies currently exist connecting type III IFN with this isotype switching. Therefore, a similar investigation into IgA2 (n=97) confirmed in SARS-CoV-2 infection that IgA2 anti-SARS-CoV-2 in severe disease correlated with ecDNA [167]. Syncytia formation and NETosis are more likely in the formation of immune complexes as an imbalance due to or caused by coagulopathy and immunothrombosis [127,164]. Other researchers examined the role of endothelial cells in both animal studies and in humans. It is presently unlikely that endothelial cells are directly infected, however studies do show colocalization with CD31 within a disrupted and inflamed endothelial layer as clearly seen by upregulation of many adhesion molecules like Platelet Endothelial Cell Adhesion Molecular-1 (PECAM-1 or P-selectin) and chemotactic factor release of CXCL10 alongside IL-6 [168,169]. Additional factors involved in platelet coagulation are vWF with adhesion molecules P-selectin and E-selectin upregulation observed as elevated in chronic COVID-19 disease patients which are known factors in endothelial dysfunction [74,170]. Therefore, Kuchroo et al performed a multiscale PHATE study (n=168) of infected SARS-CoV-2 patients that differentiated between neutrophil and monocyte populations respectively $CD16^{hi}$CD66b/

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D14−CD16^{hi}HLA-DR^{lo} monocytes markers to find that there is a T helper 17 (T_H17) cell response generating IFN-γ and granzyme B [171]. In this key finding CD14$^-$CD16^{hi}monocytes were enriched in severe infection, and it was confirmed that HLA-DR upregulation correlated with severity. In 2020 it was shown in (n=102) chronic COVID-19 disease patients higher serum levels of these cytokines IL-2, IL-4, IL-6, IL-10, TNF-α, IFN-γ with C-reactive protein (CRP) correlating with IL-10 [50]. IL-1 is a key cytokine involved with neutrophil activation which shares homology and similar functions with TLR families as above [62,172]. IL-1α and IL-1β have been implicated in coronavirus disease through other studies. Recent research has investigated autoantibodies to IL-1RA that were present in 62% (13/21) samples of multisystem inflammatory syndrome in children (MIS-C) with further research ongoing [173]. Recently immunohistochemical analysis (n=60) showed that endothelial cells express IL-1β in lung samples obtained during COVID-19 disease [172]. IL-1β and IL-6 are pro-inflammatory cytokines thought to be responsible for oedema-associated damage to type II pneumocytes by changes to decreased production of pulmonary surfactant and increased production of other mediators which stimulate homing of MΦ and neutrophils to the lungs by chemotaxis[174]. Initially it was proposed that immunity could be divided into 3 types in short IFN-γ producing cells (NK cells, T_c and T_H1) regulating activation of monocytes, secondly T_c and T_H2 regulation of mast cells, basophils, eosinophils through IgE with a third type mediated by RORγt$^+$ T cells and T_H17 regulation of monocytes and neutrophils [68,175]. Alberca et al examined a novel cell sub-type defined as myeloid derived suppressor (MDSC) cells within laboratory case studies: CD33$^+$CD11b$^+$HLA-DR$^-$CD14$^-$CD66b$^+$ and CD33$^+$CD11b$^+$HLA-DR$^-$CD14$^+$CD66b$^-$ cells in peripheral blood have been shown to be markers of chronic COVID-19 disease to correlate possible MDSC and polymorphonuclear which have been seen to increase in in chronic inflammation[176].

7. Monocytes and Macrophages

i) Monocyte Development

Since the advent of flow cytometry and discovery of monocytes by Ehrlich and Metchnikoff, currently identified monocyte sub-sets are defined by classical (CD14^{++}, CD16$^-$), intermediate (CD14$^+$, CD16$^+$), and non-classical (CD14$^+$, CD16^{++}) markers [177]. Monocytes represent around 10% of the leukocyte population and are short lived (1-2 days). circulating in blood, bone marrow, and spleen. In keeping with other cells, also undergoing apoptosis to form a cluster of antigen presenting cells (APC) including dendritic cells (DC), and macrophages (Mϕ) developing during inflammatory insult. By phagocytosing viral antigens alongside antigen presentation (using MHC class II) as well as synthesizing and secreting cytokines production monocytes are therefore central to the immune response. Monocytes produce either or both cytokines' chemokines and develop into inflammatory DCs or macrophages with development affected through TLRs and PAMP stimulation. As mentioned earlier, monocyte expression of MHC class II molecules (HLA) affect antigen presentation to T cells. In response to stimuli, monocytes have a differential response and in vitro stimulation found classical monocytes CD14^{++}, CD16$^-$, did respond to IFNγ, GM-CSF and IL-4 and that intermediate monocytes could change MHC class II (HLA-DM) presentation which associates with TCR but also that GM-CSF can modify MHC class II HLA-DR on CD14$^+$CD16$^-$monocytes which are suggested to the predominant inflammatory subset with hypotheses that non-classical CD14$^+$, CD16^{++} monocytes could be involved in patrolling endothelial vessels and being affected by IFN-γ changes. Importantly IL-10 can downregulate MHC Class II on monocytes but specifically HLA-DR on all monocytes and HLA-DM on CD14$^+$, CD16$^+$[178]. Alternative[178]. Classical monocytes can secrete proinflammatory cytokines such as IL-6, IL-8, and chemokines CCL2, CCL3, and CCL5. Throughout monocyte development progressive changes in activation and upregulation of CD16 expression can occur with antigen presentation complexes would usually occur accompanied by cytokine secretion of TNF-α, IL-1β and IL-6 upon TLR stimulation. Therefore COVID-19 case studies below will clarify context of these unique cells.

ii) Monocytes in SARS-CoV-2 Infection

In COVID-19 disease it was intimated that classical CD14^{++}CD16$^-$ monocytes were a source of upregulated chemokine CCR2 along with a neutrophil chemoattractant IL-8, TNFα and upregulated gene expression synthesis of IL-1β and IL-18 with less CD14$^+$CD16^{++} monocytes confirmed with downregulation of HLA-DR in severe patients (n=12) as above. [179,180]. These cellular populations are further characterized by CD195 (CCR5), as well as TNF-α receptors CD120a/CD120b (TNFR1/2). Both these receptors were found in blood serum and up-regulated along with ADAM17 known to affect L-selectin (CD62) shedding [181]. Other soluble shed markers of immune cells were measured

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in sera being sCD14 and sCD163 and although unrelated to severity correlated with standard blood sera proteins (acute phase, ferritin, LDH, CRP, procalcitonin) [182]. In addition, CCR5 inhibition studies, during prolonged SARS-CoV-2 infection and disease, demonstrated that changes to CD14/CD16 subsets did occur and affected pro-inflammatory cytokines alongside $CD4^+/CD8^+$ T cells reduction. These researchers showed that IL-2, IL-4, CCL3, IL-6, IL-10, IFN-γ, and VEGF were elevated but that also T_{reg} cells were reduced with concurrent GM-CSF reduction which could affect monocyte development as above.[183]. Therefore, as with NK cell FACs analysis (utilized in NK cell classification) other researchers looked differentiating these $CD14^{high}CD16^-$ monocytes by CD16 markers to find occurrence via inflammasome activation (NLRP3) evidenced by caspase-1 activity in severe COVID-19 disease which concurred with dysregulation of mitochondrial superoxide and lipid peroxidation markers of oxidative stress, and this was later confirmed with gasdermin D cleavage studies. *In vitro* studies showed this can be causal in IL-1β secretion by SARS-CoV-2-exposed monocytes [184]. Specifically, the numbers of circulating classical monocytes ($CD14^{++}CD16^-$) decrease, but the numbers of intermediate ($CD14^+CD16^+$) and non-classical ($CD14^+CD16^{++}$) monocytes increase [185]. Alternative transcriptomic analysis confirmed that $CD14^+CD16^+$monocytes displayed and induced temporal inflammatory gene signature (ISG) but only in acute SARS-CoV-2 infection (IRF7, OSG15, IFI44L, IFIT1 and IFIT3) but IL-8 and IL-1β with CCL3 were substantially upregulated without induction of pro-inflammatory cytokine genes like TNF, IL-6, IL-1, CCL3, CCL4 or CXCL2 in these cells that showed reduced HLA-DR expression and resulting reduced antigen presentation capability [186].

iii)Macrophage Introduction

In the 1950s- 1970s macrophage (Mϕ) metabolic cycles were closely examined in what was then known as the Warburg effect with macrophages in tumours seen to change metabolic profile and indeed recently researchers suggest that activation of macrophages or DCs with a range of stimuli ((LPS, TLR3 ligand poly(I:C), type I IFN)) that all induce a metabolic switch from oxidative phosphorylation (OXPHOS) to glycolysis with resultant reduction in the TCA cycle, while lactate production drives macrophage metabolism and flux through the pentose phosphate pathway upwards [187]. Shortly after. Mϕ are tissue present phagocytic cells (that include brain microglia, liver Kupffer cells and others) and are the most abundant immune cell type within the lung and are classified as AM or interstitial Mϕ (IMs). Mϕ characterization has evolved to differentiate between varying inflammatory phenotypes commonly referred to as M0 (non-activated), M1 (pro-inflammatory) and M2 (anti-inflammatory) by polarization and cytokine secreted, however are currently not defined by CD nomenclature [142,143]. As M-CSF and GM-CSF induce differentiation it was suggested that Mϕ are subdivided into M1 secreting cytokines IL-1β, IL-6, IL-12 and TNF-α with M2a TGF-β, M2b IL-10 IL-1RA and M2c IL-10 respectively with M2a Mϕs being profibrotic that induce a T_H2 type response [144]. M2b macrophages are involved with T_H2 activation or regulation with and M2c macrophages are involved in immune suppression, tissue repair, and remodeling. IFN-γ is thought to polarize M1 macrophages causing up-regulation of inflammatory cytokines upon viral infection whilst inhibiting growth but enhancing apoptosis of lung cells in vitro (Lian et al., 2022). AM regulate lung pulmonary immune responses. They are distinct by ability to induce and inhibit inflammatory responses on exposure to pathogens and cell surface markers [174].

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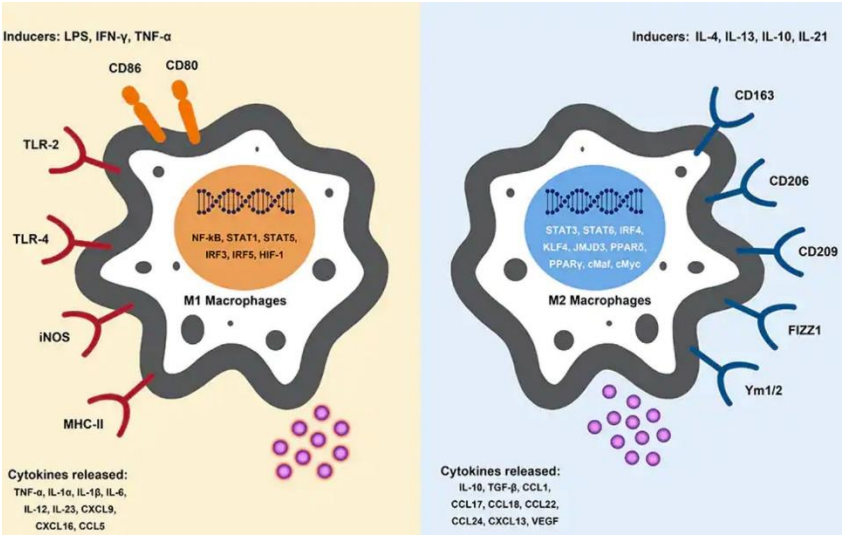

Figure 4: ***Functional subtype diversity of Macrophages*** [174]

Dysregulation of AM polarity is pivotal in the pathogenesis of respiratory diseases such as silicosis [188]. The AM/M1/M2 gene markers and protein markers within bronchoalveolar lavage fluid (BALF) can be used to assess polarity state alterations associated with diseases. Staining methods such as hematoxylin/ eosin and trichrome staining are utilized on experimental lung tissue clinical samples. M1 Mϕ are consistent with pro-inflammatory responses, pathogen resistance and their polarity is prioritized during early stages of disease. Antagonistic M2 cells typically occur at sites where anti-inflammatory microbial effects occur, tissue remodeling is required around epithelial cell layers [188]. A hybrid M1/M2 phenotype allows for rapid switching between desired functions appropriate in response to presenting stimuli and changes within healthy mucosal tissue environments [189]. Noteworthy, the dichotomy of steady state polarization is not well understood. However, scientists dictate that steady-state M1/M2 phenotypes appear stable following exposure to in vivo bacterial and viral agents **(ref#2).** This was confirmed by **c**omparative studies between UK and Malawi cohorts revealing similar AM surface pattern recognition markers. The majority of AM were classified as $CD206^{hi}CD86^{hi}$. The respective M1/M2 like phenotypes ($CD206^{lo}CD86^{hi}$) and ($CD206^{hi}CD86^{lo}$) represented less than 1% of the entire AM cell population. $CD206^{hi}CD86^{hi}$ subtypes possessed greater CD163-M2 marker levels, comparative to M1 and M2 subpopulations. Analytical experiments also found CD80 and CD64 M1 markers and HLADR activation markers were greatest expressed in $CD206^{hi}CD86^{hi}$ compared to other AM subsets **(See Figure 4).**

iv) Macrophages role in SARS-CoV-2 Infection

Recent studies appear to clarify that M0/ M1 macrophages can express CD68 and CD80 with CD163 as a highly specific mannose receptor expressed in M2 macrophages. *In vitro* studies show CD68, CD80 and CD163 were expanded in SARS-CoV-2 samples indicative of expansion of both M1 / M2 macrophage phenotypes but importantly. no difference in $CD206^{+}HLA\text{-}DR^{-}$ or $CD206^{-}HLA\text{-}DR^{+}$ MΦ as AM/ IM populations[190]. HLA-DR encoded on chromosome 6 region 6p21.31 presents S protein antigens either or as combinational peptide units of S1/S2/RBD, so this represented a key finding that antigen presentation was not occurring by this cell type [190]. Interestingly Mϕ and MDSC (myeloid derived suppressor cells) expressing CD68 and CD163 were looked at in 2018 in the context of thrombocytopenia (ITP) to try and clarify MDSCs characteristics further with indications that CCL2/CCL3 and eotaxin but also IL-1ϐ may expand both these cell types within ITP patients [191] Mϕ subpopulations can be further characterized by HLA-DR, CD195 (CCR5), and TNFR1 / TNFR2 expression, which is higher on intermediate monocytes, followed by classical and then non-classical monocytes as well as MΦ[192]. A recent preprint suggests that within acute SARS-CoV-2 infection monocytes change their inflammatory gene signature (ISG) from innate immune functions as $CD14^{+}$ monocytes developing into pro-thrombotic showing differential upregulation of MHC Class II along-side MHC class I downregulation (HLA-DR/HLA-ABC) with accompanying gene signatures downregulated that would affect IFN production (e.g. IFNA1,IFNA2) ,but also TLR7 and AIM 2 as mentioned below affecting increased expression of pathways involved in hemostasis and immunothrombosis [193]. In contrast, TNFR2 is expressed at

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high levels on non-classical monocytes, followed by intermediate, and then lowest expression on classical monocytes [194]. Enlarged monocytes with M2 Mϕ characteristics can secrete IL-6, IL-10, TNF-α and express CD11b^{+}, CD14^{+}, CD16^{+}, CD68^{+}, CD80^{+}, CD163^{+}, CD206^{+}/CD14^{high}CD16^{-} displaying inflammasome activation evidenced by caspase-1/ASC-speck formation in severe COVID-19 disease when compared to mild or healthy controls [[184]. AM are polarized towards M1/M2 according to the microenvironment existence and required immunological responses. It is established that M2 Mϕ are Th2 like and can produce allergic cytokines which are related to tissue remodeling and pathology that include IL-4/ IL13 and histamine H1 Mϕ receptor but also eosinophil H4 effects[195]. CD61 and CD163 increase in severity alongside CD163 and Tregs. It is possible that M2 Mϕ, together with suppressor Tregs cells, promote the immunosuppressive environment.CD163 and CD206 are major markers for the identification of M2 Mϕ. Related surface markers for M2 Mϕ also include CD68. Compared with CD68, CD163 is more selective to Mϕ, so CD163 can be used as a highly specific marker for M2-type Mϕ. In particular, the numbers of circulating classical monocytes (CD14^{++}CD16^{-}) decrease, but the numbers of intermediate (CD14^{++}CD16^{+}) and non-classical (CD14^{+}CD16^{++}) monocytes increase with single-cell transcriptomic analysis of PBMC in 7 hospitalized COVID-19 patients revealing a depletion of CD16^{+} monocytes in peripheral blood and the induction of an ISG signature in CD14^{+} monocytes, but detecting no substantial induction of pro-inflammatory cytokine genes, such as TNF-α, IL-6, IL-1β, CCL3, CCL4 or CXCL2 in these cells [185]. Therefore, concurrent *in vivo* research shows that E protein could suppress inflammasome priming and NLRP3 inflammasome activation and reduced expression of pro-IL-1β, levels of IL-1β and IL-18 in BALF, with Mϕ infiltration in the lung through NLRP3/caspase-1/IL-1β signaling [196]. However, M1 Mϕ pro-inflammatory phenotype are defined by IFN-γ and lipopolysaccharide or to M2 Mϕ anti-inflammatory phenotype by IL-4 to M1 Mϕ that have a lower endosomal pH, favoring membrane fusion and allowing the entry of viral RNA from the endosomes into the cytoplasm, where viral replication packaging and release can occur. In contrast, M2 Mϕ s have a higher endosomal pH but a lower lysosomal pH, thus delivering the virus to lysosomes for degradation. In hACE2 transgenic mice distinct uptake, amplification, and release of SARS-CoV-2 by M1 and M2 Mϕ with preferential increased viral loads within M1 Mϕ but also less nucleocapsid expression within M2 Mϕ [197]. Related surface markers for M2-type cells also include CD68. Compared with CD68, CD163 is more selective to MΦ, so CD163 can be used as a highly specific marker for M2-type Mϕ. Interestingly recent research indicates little phenotypic difference between either alveolar or interstitial Mϕ based on CD206^{+}HLA-DR^{-} or CD206^{-}HLA-DR$^{+,}$ expression but that M1 Mϕ IL-1β, IL-18 along with CXCL9, CXCL10, CXCL11, CXCL16, together with CCL2 and anti-inflammatory M2 Mϕ expressing TGM2, APOE, A2M, CCL13, CCL26, with TREM2 tent towards toxicity of M1 Mϕ that all have affinity for CXCR3 receptors. Therefore, as mentioned prior intracellular inflammasome suppression or activation can be seen in sequencing studies and confirmed activation of caspases (*CASP3*, *CASP 8*, *CASP 10*, and *APAF1)* that are upregulated in iM1ϕs or iM0ϕ and undergo apoptosis within 48 hours during infection [190].

Conclusions are difficult to draw with how macrophages interact with B and T cells. The complexity of AM has many researchers baffled as noted by nasal pneumococcal colonization disrupting CD4^{+} T cell responses but having no effect on key M1 and M2 polarization surface markers. [189]**.** Therefore, viral shedding, solubility incompetent modulatory polarization, affects Mϕ and is transient and reversible Prescribing synthetic and natural M1 Mϕ polarization inhibitors has been theorized to decrease disease severity and risk of associated mortality stemming from host reactions in COVID-19 disease**.** Indeed many anti-inflammatory therapeutics like methylprednisolone and dexamethasone were utilized that preferentially modulate AM polarization in excessive inflammatory responses and preserve lung oxygenation levels within patients [174]. M1/M2 polarization are key indicators in the progression and management of severe COVID-19 disease but is also evident in other pathologies like Ebola [174]. The importance optimal polarity may have on maintaining homeostasis between protective immunity and immune responses within alveolar, gastrointestinal, and additional mucosal spaces also warrant further investigation in SARS-CoV-2 therapeutic clinical trials [189]

7. Natural Killer Cells

i) Introduction

Natural killer (NK) cells are cytotoxic lymphocytes that provide innate immune defence against viral infections and cancer representing around 10% of circulating leukocytes. NK cells were originally classified in 1989 and later phenotypically into CD56^{bright} CD16− , forming most NK cells and a further three including CD56^{bright} CD16^{dim,} CD56^{dim} CD16^{-}, CD56^{dim}CD16^{bright}, and CD56^{-} CD16^{bright} with the latter CD56^{-} CD16^{bright} NK cells expanded in other pathologies like human

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immunodeficiency virus and cancer [198–200]. Initially NK cells were described as sensitive to IL-2-dependent activation and proliferation and characterized by CD56 / CD16 markers with TNF-α equally found with IFN-γ preferentially produced in the $CD56^{bright}$ NK cell [198]. $CD56^{dim}$ $CD16^{bright}$ NK cells represent 90% of the total NK cell population. $CD56^{bright}$ NK cells were found to express CD2, CD11c, CD44, CD49, CD54 and CD62L, with $CD56^{dim}$ NK cells expression of CD11 distinguishing respective differences with $CD56^{bright}$ subset preferentially migrating to secondary lymphoid organs with the $CD56^{dim}$ cells to inflammatory sites [199]. Research evolved and clarified the role of CD16a as a potent cytotoxicity receptor on human natural killer (NK) cells and CD16a has affinity for IgG1 and IgG3. Therefore, antibody recognition via opsonization of IgG targets can occur via CD16a recognition of IgG-opsonized targets. NK cells trigger downstream potent cytotoxic mechanism called antibody-dependent cell-mediated cytotoxicity (ADCC), and independent mechanisms leading to cell death utilizing lytic granules dependent or independent of perforin and granzyme B release to lyse the cell target [201–203]. However, NK cell receptors also possess independent ligands that activate target killing, independent of lytic granules, such as Fas ligand and TRAIL which may activate NK cell cytotoxicity. Furthermore, recent clarification of the enzyme caspase-8 intertwining the three major cell death pathways, including apoptosis, pyroptosis, and necroptosis is suggestive given recent research indicating its role in both T cells and NK cells [204]. Much is unknown about NK cell antigen presentation, however recent articles are suggestive from in vitro studies that increased HLA-DR expression is associated with NK cell proliferation activity, and IFN-γ production with higher expression of CD86 and NKG2D [205,206] Therefore, NK cells are currently identified as lymphocytes expressing CD56 although other researchers did indicate that NK cell CD7 and CD4 can be expressed separating these from other antigen presenting cells like dendritic cells and monocytes.

ii) NK Cells in SARS-CoV-2 Infection

During SARS-CoV-2 infection and as NK cells are present in higher levels in the lung BALF analysis it was shown that $CD56^{bright}$ and $CD56^{dim}$ NK cells displayed an activated effector phenotype with $CD56^{bright}$ NK cells exhibiting higher levels of granzyme B, CD25, HLA-DR, and Ksp37 all necessary for cell mediated lysis, or antigen presentation [207]. Furthermore, this was corroborated by other studies demonstrating that NK cells as measured by $CD56^{+}CD57^{+}$ and PD-1 were present at higher levels in naïve individuals [208]. Indeed, higher levels of soluble CD25 at the same-time were found in a BALF analysis (n=280) indicating this is driven by the delayed clearance of SARS-CoV-2 infection and expansion of $CD25^{+}PD\text{-}1^{+}$ $CD8^{+}$ T cells[209] . Very recently, although not peer reviewed, immune cell profiling PD-1 was confirmed to be preferentially expressed with downregulation of HLA-DR in a sampling (n=215)[210]. Therefore, impeded antigen presentation could potentially occur but importantly PD-1 can affect differentiation of $CD8^{+}$ T cells. Interestingly NK cells are known to utilize MHC Class I HLA-E to present antigens and recently the effect of SARS-CoV-2 nsp13 was seen in vitro to

8. T Cells

i) T Cell Introduction

T cells are thymus derived lymphocytes that provide adaptive immune defence representing 10-20% of circulating leukocytes and 2–3% in human peripheral blood. Research from 1972 evolved to characterize these and thereafter with CD4 discovery in 1984 and into phenotypes to include T_H1, T_H2, T_H17, T_{reg}, T_H9, T_C, T_H22. with various developmental characteristics and markers that are defined through CD nomenclature, cytokine regulation and chemokine receptors. Development of T cells occurs in bone marrow lacking characteristic $CD4^{+}$ and $CD8^{+}$ receptors undergoing SHM and selection processes that generate $CD4^{+}CD8^{+}$ double positive (DP) thymocytes or others that include $CD4^{+}$ or $CD8^{+}$ single positive (SP) thymocytes that ultimately emerge into the periphery as T cells generally exhibit a $CD45RA^{+}CCR7^{+}$ phenotypes although CCR7 is also expressed on DCs. Comparatively recent discovery of T_{REG} cells by Sakaguchi in 1995, defined with clarification that $CD4^{+}CD25^{+}$ cells express a nuclear transcription factor forkhead box P3 (FoxP3). T cells undergo clonal expansion where activation/differentiation occurs into effector T cells that mediate pathogen clearance after which T cells undergo apoptosis whilst memory T cells persist. Cytotoxic $CD8^{+}$ T cells are known to use a variety of proteolytic enzymes including granzymes and, whilst $CD4^{+}$ T cells regulate maintenance through T_H1 and T_H2 lineages in concert with this $CD8^{+}$ response via regulation of exhaustion and antigen recognition. T cells are further defined as naive $CD3^{+}$ $CD4^{+}$ $CD45RA^{+}$ $CCR7^{+}$ (T_N) which differentiate into central memory (T_{CM}: $CD45RA^{-}CCR7^{+}$), effector-memory (T_{EM}: $CD45RA^{-}CCR7^{-}$), stem-cell memory (T_{SCM}: $CD45RA^{+}CCR7^{+}CD95^{+}CD122^{+}$) but also peripheral resident memory (T_{RM} CD69, CD103, CD49) T cells respectively [211]. T_{RM} cells produce key cytokines IFN-γ, IL-17, TNF-α, and IL-2 can express PD-1, LAG-3, and CTLA-4. In serum CCR7

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distinguishes T cells homing to lymph nodes (LN) when expressed, or effector memory (T_{EM}) subsets migrating to tissues when absent. However as above where we discussed DN B cells, DN T cells which do not express CD4 or CD8 are known to exist. [212]. Many complicated terminologies exist referring to many proteins in this paper, so we will update this part over the coming weeks with full detail on T cells.

ii) T cells in SARS-CoV-2

COVID-19 vaccine immunogens logically exclusively focused on S protein nAb responses, with little interest in overall cellular immunity. Interestingly, there is data accumulating which suggest that T cells perform a key role in vaccine protection against chronic COVID-19 disease, and against recent variants of Omicron lineages (BA1, BA2, BA4/5 and more recent BQ1) that display further epitope escape from recognition by nAbs. Epitopes represent the unique protein sequence that either infection present proteins or vaccine immunogens recognize. These observations should have an impact for using current COVID-19 vaccines and for the development of next-generation vaccines against COVID-19 and other infectious diseases. Early in the pandemic in 2020, case reports appeared from Italy and India of people with a rare disorder showing deficiency in antibody production [213] or even no B-cells at all (agammaglobulinemia, XLA). Routine surveillance in this risk group identified two XLA patients who developed COVID-19 while getting Ig infusions. These patients indeed developed pneumonia which eventually resolved but never required ventilation or intensive care. This was the first time that B-cell response might be considered important but is not strictly required to overcome COVID-19 disease [214,215] Also in 2020, groups in Germany/USA independently discovered reactive T cells to S protein of SARS-CoV-2 in around 30% of healthy donors when analyzing the T cell response of COVID-19 patients [216,217]. These T cells belonged to the $CD4^+$ lineage and were primarily directed against C-terminal epitopes of S protein, that has higher homology amongst spike glycoproteins of human coronaviruses (hCoVs) than the N-terminal region. Braun et al. further showed that these cross-reactive T cells were functional against spike's C-terminal part of the human endemic coronaviruses hCoV-229E and hCoV-OC43, as well as that of SARS-CoV-2 [217]. Daniela Weiskopf's group did map 142 T cell epitopes across the SARS-CoV-2 genome by using pre-pandemic blood samples to simplify the exact investigation of the SARS-CoV-2-specific $CD4^+$ T cell repertoire. They demonstrated an array of preexisting memory $CD4^+$ T cells with cross-reactive capability with equal affinity to SARS-CoV-2 and the common cold coronaviruses hCoV-OC43, hCoV-229E, hCoV-NL63, and hCoV-HKU1 [216]. As COVID research continued, all B and T cell markers CD3/CD4/CD8/CD19 were re-profiled as above and below. Therefore, early in 2020, in a cohort of 187 samples (patients) indications of both B and T cell subset differences showed an overall 50% lymphocyte cell count reduction with resulting examination of initial characteristic cellular markers of $CD4^+$ T-cell $CD8^+$ T-cell B-cell and NK-cells to find in chronic COVID-19 disease there was a further 33% reduction in comparison to acute COVID-19 infection which was indicative of T cell function being affected. [218]. Shortly after (as in Table 3) further clarification elucidated this further to show within T suppressor cell populations possessing the T cell activation marker CD28. Furthermore, CD28 requires co-stimulatory molecules CD80, CD86 of which CD80 is present on B cells, dendritic cells, T cells and macrophages (that requires co-stimulatory molecules (CD80/CD86). Therefore, within chronic COVID-19 disease there are changes within the T cell subsets and it is of note that within T cell subsets as with B cells there are also DN T cells but also recognition of SARS-CoV-2 by cross-reactive T cells as above.

SARS-CoV-2 reactive T cells were found in peripheral blood and tonsil from donors unexposed to SARS-CoV-2 [219–227]. A number of these samples were analyzed by reports prior to the COVID-19 pandemic [219,220,222,223,226,227], eliminating the likelihood of exposure of the donors to SARS-CoV-2 infection. An explanation to this observed phenomenon is that we are observing a recall response of SARS-CoV-2–cross-reactive memory T (T_{mem}) cells that were created upon encounter with homologous proteins derived from prior coronaviruses. Before SARS-CoV-2, 6 hCoVs were circulating and SARS-CoV and MERS-CoV sharing most homology to SARS-CoV-2 and causing respiratory syndromes [228]. It was reported by Le Bert et al. that in blood samples from convalescent SARS-CoV-1 more than 17 years ago that T cells were detected recognisingSARS-CoV-2 [226]. Nevertheless, for SARS-CoV and MERS-CoV the dissemination was more restricted than for SARS-CoV-2, meaning that it is unlikely to find cross-reactivities over a wide range of the population. On the other hand, the 4 human coronaviruses (hCoV-OC43, hCoV-HKU1, hCoV-NL63, hCoV-229E) circulate each year causing the common cold. These hCoVs share less homology with SARS-CoV-2. And indeed, it could be demonstrated that cross-protective T cells epitopes that stemmed from common cold hCoVs could recognize SARS-CoV-2 [222,229–231].

Though, it is still debated and far from being clear whether common cold hCoVs-mediated cross-reactive T cells are a benefit in the host defense against COVID-19 disease or not. Interestingly,

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in 20% of SARS-CoV-2–naïve donors and a higher percentage of infected or vaccinated donors a report determined that SARS-CoV-2 peptide S816-830, a relatively conserved protein in common cold hCoVs, was to activate $CD4^+$ T cells, which led the authors to the conclusion that cross-reactive $CD4^+$ T cell response might be protective in COVID-19 [229]. In contrast to these findings a different group found the same epitope activated $CD4^+$ T cells in more donors with breakthrough infections after vaccination compared to vaccinated donors with no breakthrough infection [231]. Moreover, it was reported by Bacher et al. that $CD4^+$ T cells cross-reactive to SARS-CoV-2 in individuals not exposed to the virus displayed low avidity, which was also found to be the case for individuals with severe COVID-19[225]. Even though these cross-reactive $CD4^+$ T cells could be determined *in vitro* by SARS-CoV-2 proteins and led to proliferation of the concomitant T-cell clones being stimulated by SARS-CoV-2, the relevance of these findings is far from being understood.

Interestingly, not only hCoVs induce cross-reactive T cells to SARS-CoV-2 but also commensal bacteria [232,233]. In COVID-19 patients as well as in healthy controls a public T_{FH} clonotype was detected that fits to SARS-CoV-2 S870-878 protein and shares homology with a symbiotic bacterial antigen. Its abundance was found to be higher in patients with acute compared to chronic symptoms than in individuals with severe symptoms, leading to the assumption that this clonotype might be protective [232]. Unexpectedly, also the old vaccine strain BCG displayed homology with 8 epitopes of NSP3 from SARS-CoV-2. Eggenhuizen et al. demonstrated that *in vitro* stimulation with SARS-CoV-2 proteins of BCG primed $CD4^+$ and $CD8^+$ T cells led to enhanced cytokine production and proliferation in an HLA dependent fashion. This mechanism might provide a partial explanation to the observation that BCG vaccination exerts some protection from COVID-19[234]. Nonetheless, it needs further studies to elucidate the features of cross-reactive T cells and the parts they play against SARS-CoV-2. induced COVID-19 disease.

n=452	Cluster of Differentiation Markers	Acute	Chronic
T cells & B cells & NK cells		↓	↓ ↓
B cells	CD3− CD19+		
T cells	CD3+ CD19−	↓	↓
NK cells	CD3−/CD16+ CD56+		↓
		Acute	Chronic
T helper	CD3+ CD4+	↓	↓
T cytotoxic	CD3+ CD8+	↓	↓
T naïve	CD3+ CD4+ CD45RA+		
T Memory	CD3+ CD4+ CD45RO+		
T Suppressor	CD3+ CD8+ CD28+	↓ ↓	↓ ↓
Activated T cells	CD3+ HLA-DR+		
Activated Suppressor	CD3+ CD8+ HLADR+		
Regulatory	CD3+ CD4+ CD25+ CD127low	↓	↓
Naïve Regulatory	CD45RA+ CD3+ CD4+ CD25+ CD127low	↓	↓

Table 4: T Cell Expression Markers (n=452) Adapted from Qin et al [235]

iii) Cytotoxic T cells:

Therefore, it can be seen above that cytotoxic T cells expressing CD28 were downregulated, but also determined that cytotoxic Tc cells ($CD8^+$) expressing PD-L1 and $CXCR3^+$ were associated with increased survival in SARS-CoV-2 infected patients. During SARS-CoV-2 infection, there was also a significant increase in terminally differentiated T_{EM} cells (T_{EMRA}) and T_{EM} localizing with T_{CM} (CCR7+ CD45RA−) differentiating into T_H2 like secreting IL-5 with T_{CM} expressing these chemokines CX3CR1, CCR6, CXCR6, and CXCR3 that represent lung-homing receptors), and that T_{CM} could express TNF-α, but also SARS-CoV-2 CD4 S protein specific T cells displayed a T_{CM} phenotype with

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specific $CD8^+$ cells heterogenous towards T_{EM}, T_{EMRA}, T_{SCM} [236]. Therefore, as lymphopenia can occur and potential insufficient antibody responses in SARS-CoV-2 infection, it is necessary to look at dysfunction inside and outside GCs in COVID-19 disease [237]. Further clarification occurred that no change in CD8 T cells ($CD3^+$ $CD19^-CD4^-$) occurred but significant increase in in $CD3^-CD19^-$ monocytes implying impaired T_{FH} cells producing IFN-γ and IL-21, with the latter critical for B cell proliferation and differentiation. Clarified as well through stimulation of these T_{FH} cells by agonism of the ICOS receptor by an antibody stimulating generation of IFN-γ/IL-21 in hospitalized COVID-19 subjects. $CD3^+$ $CD4^+$ $CXCR5^+$ $ICOS^+$ lymphocytes within ambulatory subjects and encompassed on average 15% of the peripheral blood CD4 compartment relative to the 8% T_{FH} population [238]. Therefore, it was further clarified that no change in CD8 T cells ($CD3^+$ $CD19^-CD4^-$) occurred but significant increase in in $CD3^-CD19^-$monocytes and implying impaired T_{FH} cells producing IFN-γ and IL-21, the latter is critical for B cell proliferation and differentiation. Clarified it as well was shown that stimulation of these T_{FH} cells by agonism of the ICOS receptor by an antibody stimulating generation of IFN-γ/IL-21 in hospitalized COVID-19 subjects. $CD3^+$ $CD4^+$ $CXCR5^+$ $ICOS^+$ lymphocytes within ambulatory subjects and encompassed on average 15% of the peripheral blood CD4 compartment relative to the 8% T_{FH} population [238]. Reports are conflicting around $CD3^+CD8^+$ cells expressing CD38 that downregulate MHC class II (HLA-DR) during SARS-CoV-2 infection although others do indicate that this correlates with disease severity [239,240]. In some cohort studies within immunocompromised patients, it does however appear that the different immunogens evoke individually different responses that could vary by underlying comorbidity. For example, compared to healthy controls which would express B Cells ($CD38^+CD19^+$) and T cells ($CD8^+$ $HLA\text{-}DR^+CD8^+$) weeks after 2 doses of vaccine (n=42) in comparison in transplant patients little change was seen in cells expressing $HLA\text{-}DR^+$, $CD38^+$, and $PD1^+$ but in immunocompromised in 13.3% no IgG S protein specific response occurred [241] .

iv) . Helper T cell

Historically T cells were classified as T_H1 and/or T_H2 types or rather $CD4^+$ and $CD8^+$ with HIV infection allowed increased understanding CD4+ receptor mediated entry some 30 years ago now, however between 2000-2014 discoveries and classification of chemokines as predominant homing ligands and receptors occurred of key relevance to the overall context of T cell development, proliferation and effector functions. Gil-Mansos group recently did a comprehensive FACs analysis comparing a control v covid group (=51) within recovered COVID-19 individuals to demonstrate a significant cluster at 10 months of recovery of $CD4^+$ $CD45RA^-$ $CCR4^-$ $CCR10^-$ $CD27^+$ $CCR6^-$ $CXCR3^+$ $CD127^+$ inferring that there is a response within the circulating $T_{FH}1$, plasmablast, and follicular dendritic cells (foDC) axis of note showing that these $T_{FH}1$ cells within the SARS-CoV-2 group did express $ICOS^+$ and PD-1+ which is relevant as T_{FH} and B lymphocyte impairment within follicular zones or extra-follicular areas outside the GC can occur . Therefore a potential imbalance within circulating memory Th subsets during acute COVID-19 was seen to polarize T_{FH} lineages leading that could potentially dysregulate the B cell response outside GC and therefore this was quantified in a cohort (n=78) to find $CD4^+$ T cells tendency to T_H2-like phenotypes in chronic SARS-CoV-2 infection that increase in $CD45RA^+CD62L^-$ (T_{CM}) and $CD45RA^-CD62L^-$ (T_{EM}) compared to a decrease in CM $CCR6^+$ T_H17-like cells [242].

9. Limitations

There is a requirement to acquire more data to sharpen the clarity of understanding of cellular and humoral immunity on a global scale. which may require revisiting and expanding on these case studies in future times.

10. Discussion

In the present COVID-19 pandemic, a crucial question that is partially resolved is whether SARS-CoV-2 infection or COVID-19 disease results in long-term protection. Analyses of B cell responses up to one year after infection show that there is a quantifiable neutralizing antibody response with memory B cells against both nucleocapsid and SARS-CoV-2 spike protein of most recovered COVID-19 patients which are stable or degrade slowly. Throughout the above case studies, virus evolution and future vaccine development do affect physiological cellular immune responses only now becoming clearer. Current SARS-CoV-2 evolution through S1 protein mutations does appear to enhance re-infection from initial Omicron variants (BA1, BA2, BA4/5) through retaining known B cell escape epitopes that include L452R, F486V, R493Q whilst acquiring other

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similar mutations (R346, N460, K444, N460) in derivative lineages, which are known to cause issues with current monoclonal antibody therapeutics [243,244]. Whilst the last paper is under peer review it remains to be seen how SARS-CoV-2 evolution will affect the overall adaptive immune system cellular T cell responses outlined above affected by ketogenesis and antigen presentation mechanisms in our next paper.

11. Conclusions

Above we have outlined relevant clinical and laboratory analysis in combination with case studies. Neutrophils are vital in pathophysiology in individuals with severe disease histories. Neutrophil accumulation through IL-6 contributes towards this. There are evidential increases in not only S protein immunogen responses but also responses to N or M protein here as clearly shown. In this article we consider cellular markers according to current immunological research that include B and T cells which are relevant to current vaccine immunogens in context. Indeed, many senior scientists both in September 2020 and in April 2022 wrote letters in this regard (Supplementary Materials) that demonstrated overall antibody positivity of 23% (NY) 18% (London) and 11% (Madrid) and is dependent on the other 10 or more T cell sub-types performing all regulatory functions of the immune system which are arguably more important in all pathologies. [245]

Supplementary Materials: The following supporting information can be downloaded at:
T cells - https://www.biospace.com/article/60-scientists-sign-letter-petitioning-fda-for-t-cell-recognition/:
Office of National Statistics COVID-19 Infection Surveys Coronavirus (COVID-19) Infection Survey, UK - Office for National Statistics; Nuffield Department of Medicine: Results - antibody positivity updates from ONS — Nuffield Department of Medicine (ox.ac.uk):

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