Salient Visionary
Publications

A Study Discovered the Role of Carbon Dioxide in the Pathogenesis of Autoimmune Disorders

Abdelrazak Mansour Ali[1*], Radwa abdelrazak ali[2] and Mohamed Abdeltawab Ibrahim[3]

[1]Department of Paediatrics, Al-Azhar University, Egypt

[2] Neuroscience Specialty, George Mason University, National Institute of Health, USA

[3]Quality management Consultant, Ministry of Health, General Director of Marsa Alam Hospital, Egypt

1. ABSTRACT

The incidence of autoimmune disorders has increased with the rise in carbon dioxide since last century.

1.1. Objective: To determine whether CO_2 is associated with autoimmune disorders.

1.2. Design: Case-control study at local tertiary hospitals in Egypt.

1.3. Methods: A total of 150 patients with various autoimmune disorders, and 75 controls were aged 20 to 70 years. The exclusion criteria were neuromuscular disorder, critical, respiratory illness, and exposure to CO_2, guided by the criteria of the National Institute for Occupational Safety. Patients recruited from November 2023 to March 2024, matched by age, sex, and other demographic variables. All participants were tested for blood gases. Certain cases were further tested to confirm autoimmune status. $PaCO_2$ analysis performed using two methods of statistical significance to validate data.

1.4. Results: $PaCO_2$ (Mean $\pm$ SD) was (48.18 $\pm$ 12.10) in autoimmune cases, compared to (42.63 $\pm$ 11.06) in control (p=0.001), number (%) of cases with $\uparrow PaCO2$=97(64.7%) for cases, and 30(40%) for control (RR=1.6167). OR (95% CI) =2.7453 (1.552 to 4.857), p=0.0005.

1.5. Conclusion: Our study confirmed a correlation between CO_2 and autoimmune disorders. The mechanism is a complex interplay between direct effect of CO_2 on cell membrane, calcium "Ca^{2+} "homeostasis and signalling pathways. CO_2 thermic effect increases the mobility of antibodies to move away from the antigens which elicited their secretion, then CO_2 protonation increases electrostatic interactions between anionic cell membranes and positive charge of antibodies, thus orienting antibodies to host antigens initiating autoimmune reaction. The significant autoimmune phenomena in skin and musculoskeletal system are due to these tissues have more cells with negative charges. Pattern of gene expression to CO_2 thermal effect imposes differences in mRNA gene translation with various phenotypic expression. CO_2 directs pathways providing benefits to autoimmunity. A new autoimmune disorder treatment is proposed. The pattern of gene expression thermal effect imposes differences in mRNA gene translation. Membrane receptors absorb heat energy reemitted from CO_2 would produce various oscillations of the electron cloud

culminating in various phenotypic expression. CO_2 directs pathways to benefit the autoimmune process. It is expectable that a new treatment of autoimmune disorders will prevail in the future.

2. Keywords: Cell; Calcium; Autoimmune; CO_2, Ca^{2+}

3. ABBREVIATIONS

Molecular Mimicry = similarities between foreign and self-peptides favor an activation of autoreactive T or B cells by a foreign-derived antigen in a susceptible individual

PIP = Phosphatidylinositol 4-Phosphate

ER/SR = Endoplasmic Reticulum/ Sarcoplasmic Reticulum

TRPCs = Transient Receptor Potential Channels

cAMP = Cyclic Adenosine Monophosphate

NFκB = Nuclear Factor kappa of B cell

RA = Rheumatoid Arthritis

SLE = Systemic Lupus Erythematosus

IBD = Inflammatory Bowel Diseases

Ig V = Immunoglobulin Variable Chain

Ag-Ab = Antigen Antibody.

RGS = Regulator of G-protein signalling

APCs = Antigen Presenting Cells

MHC = Major Histocompatibility Complex

TCRs = T Cell Receptors

ITAMs = Immune-Receptor Tyrosine-based-Activation-Motif

4. INTRODUCTION

Despite global warming being implicated in diverse pathologies, currently no studies to explore defined carbon dioxide cellular thermal damage exist. Here, we present the novel study adopting a principle of thermo-acidic impact on individual cells and explored their mediated autoimmune disorders.

CO_2 is a molecule composed of one carbon atom and two oxygen atoms, it absorbs infrared radiation in a unique manner due to its vibrational patterns of stretching and bending that can amplify absorption of radiation. Clearly, CO2 is among the most important greenhouse gases responsible for terrestrial thermal regulation and thorough knowledge of its molecular absorption profile is necessary for accurate simulations of climate change [1]. It is acidic, carried in blood in different forms, the majority of CO_2 is carried in the bicarbonate buffer system. Upon entrance into red blood cells, carbon dioxide is quickly converted to carbonic acid by the enzyme carbonic anhydrase. Carbonic acid immediately dissociates into bicarbonate and hydrogen ions. Other forms bound to haemoglobin as carbamino compounds [2,3]. Hypercapnia is increased partial pressure of carbon dioxide (Pa CO_2) above 45 mm Hg, there are several mechanisms the body should moderate CO_2 by acid-base buffering system. Retention of CO_2 in blood is an important consequence of a handful of sleep disorders such as sleep apnea, and obesity [4]. Infrared pulses are absorbed by water to produce heating that reversibly alters electrical capacitance of plasma membranes, depolarizing target cells, and triggering thermosensitive ion channels

forming membrane pores [5]. The pH of a liquid is determined by its hydrogen ion concentrations: the higher the concentration of hydrogen ions, the lower the pH value [6].

Autoimmune Disorders The increasing prevalence of autoimmune diseases has become one of the leading concerns in public health. Autoimmune diseases occur when the immune system mistakenly attacks healthy cells and tissues in the body, leading to chronic inflammation, tissue damage, and organ dysfunction [7]. The fundamental underlying mechanism of autoimmunity is defective elimination and/or control of self-reactive lymphocytes. Studies in humans and experimental animal models are revealing the genetic and environmental factors that contribute to autoimmunity [8]. Infectious diseases are commonly implicated as potential initiators of Autoimmune Diseases (ADs) and represent the most known factor in the development of autoimmunity. Epidemiological data and animal studies on multiple ADs suggest that molecular mimicry is one of the likely mechanisms for the loss of peripheral tolerance and the development of clinical disease. Other mechanisms such as defects in central tolerance, nonspecific bystander activation, epitope-determinant spreading, and/or constant antigenic stimuli, may also contribute for breach of tolerance and to the development of ADs [9]. Additionally, autoimmune disease results from an interplay between a genetic predisposition and environmental factors. Genetic predisposition to autoimmunity is complex and can involve multiple genes that regulate the function of immune cell populations. Studies showed that patients may express autoantibodies many years before the appearance of clinical or laboratory signs of disease - a period called pre-clinical autoimmunity [10].

Recently, with the advancement of understanding of the immunological and molecular basis of autoimmune diseases, gene modulation has become a potential approach for the tailored treatment of autoimmune disorders. Gene modulation can be applied to regulate the levels of Interleukins (ILs), Tumor Necrosis Factor (TNF), Cytotoxic T-Lymphocyte-Associated Antigen 4 (CTLA-4), interferon-γ and other inflammatory cytokines by inhibiting these cytokine expressions using short interfering Ribonucleic Acid (siRNA) or by inhibiting cytokine signalling using small molecules. In addition, gene modulation delivering anti-inflammatory cytokines or cytokine antagonists showed effectiveness in regulating autoimmunity. The potential target genes for immunomodulation were studied in many autoimmune diseases including Rheumatoid Arthritis (RA), Systemic Lupus Erythematosus (SLE), Inflammatory Bowel Diseases (IBD) and Multiple Sclerosis (MS) [11].

Calcium Channels & Homeostasis Cells maintain precise intracellular Ca^{2+} *via* a complex system of Ca^{2+} channels, transporters, Ca^{2+} ATPases, and signalling effectors, including specific lipid kinases, and phosphatases. Excessive intracellular Ca^{2+} caused functional defects in subcellular organelles such as endoplasmic reticulum" ER", lysosomes, and mitochondria [12].

Calcium is essential for intracellular processes, cell stored Ca^{2+} in high concentrations in (ER) or (SR) which serve as main storage site for calcium. Ca^{2+} channels include all pore-forming, Ca^{2+} permeable proteins [13]. It is demonstrated that Ca^{2+} signals encode information in frequency, kinetics, amplitude, and spatial extent [14]. One of the most important types of calcium channels are voltage-gated channels which respond to change in voltage across cell membranes and driven by an electrochemical gradient. A particularly important calcium

channel is Ca^{2+} ATPase at ER/SR membrane, called sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase [15]. When Ca^{2+} stores inside ER/SR becomes depleted, it will be replenished by special calcium channels called store-operated calcium channels, where low Ca^{2+} is sensed by a Stromal Interacting Molecule (STIMs). They interact with another protein called Orai1, referred to as a Calcium-Release Activated Channel (CRAC) [16]. STIMs act as sensors not only of decreased ER Ca^{2+} but also of temperature increases and acidosis. STIMs target proteins other than Orai channels, including voltage operated CaV1.2 channels, TRPCs, and SERCA Ca^{2+} pumps [17]. It was evidenced that altered Ca^{2+} in lymphocytes leads to autoimmune and immunodeficiency syndromes [18].

Ca^{2+} channels also generate signals that are spatial in nature and dependent on highly localized signalling structures. They may create elementary signals called calcium "puffs" or "sparks," to form organized complexes with proteins called G-protein coupled receptors or receptor tyrosine kinases [19]. Acidity can suppress anti-tumor T lymphocyte function by a significant upregulation of inhibitory immune checkpoints TIM-3, LAG-3, and CTLA-4 of T cells [20]. This acidosis-induced upregulation of immune checkpoints contributed to immune evasion and tumor progression. Extra tumoral acidity represented a mechanism of resistance to CTLA-4 inhibitors [21,22]. CTLA-4 is a critical negative regulator of autoimmune diseases [23]. The functional integrity of the CD28 molecule was necessary for CTLA-4 knockout to cause autoimmune diseases [24].
Ca^{2+} is an important cation able to function as a second messenger in different immune cells. Intracellular Ca^{2+} Signalling has been implicated in pathogenesis of autoimmune and congenital immunodeficiency disorders [25]. Dysregulated Ca^{2+} signalling is involved in the pathophysiology of autoimmune diseases [26].

5. METHODS

For our knowledge, this is the first novel study to uncover the role of CO_2 in pathogenesis of autoimmune disorders. The study approval was received from administration of Hurgada, Marsa Alam and Nasser Institute Hospitals Cairo, Egypt. Routine consents for laboratory diagnosis were implemented for all cases according to hospital regulations, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Cases were recruited from hospitals from November 2023 to March 2024. The index date for this study, was established to comply with seasonality of autoimmune diseases [27]. A total of 150 cases (77 males and 73 females) with various autoimmune disorders compared to 75 controls (40 males and 35 females) were aged 20 to 70 years. Cases and controls were matched for any potential confounders and were recruited from the same population and locality.

Selection of participants are equivalent with respect to those matching criteria based on previous knowledge of an association with the outcome of interest. Sources for cases include patient rosters at medical facilities. The comparison group ("controls") was representative of the source population that produced the cases, and sampled in a way that is independent of the exposure to autoimmune disorders. Both groups were matched to socio demographic characteristics. Eligible cases were enrolled from outpatient clinics as well as inpatient admissions after informed consent obtained. The study was approved by the medical ethical review boards of the medical

centers. Printed records were collected to verify inclusion criteria, medications given, clinical review, family and past histories as well as test results.

Selected parameters including age, sex, urbanization, social status, and comorbidities showed close balance between two cohorts. All participants had been seen for examination, including vitals, weight, height, Body Mass Index (BMI), and demographic variables gathered by questionnaire including age, sex, occupation, smoking status, family history, and history of chronic respiratory illness or occupational exposure to CO_2, guided by criteria of "National Institute for Occupational Safety and Health, August 1976". Emphasis to exclude farmworkers, mining, and industries related to CO_2 exposures was considered. Participants were free of neuromuscular disorder, critical illness, and multisystem organ failure. Participants were tested for arterial blood gases using ABL90 FLEX PLUS blood gas analyser to determine partial arterial pressure of CO_2 and stratified according to Pa CO_2 levels less than and greater than median. The considered standard range values of partial pressure of CO_2 were between 35 mmHg to 45 mmHg. Pa CO_2 was analyzed using two methods of statistical significance to validate our findings; it included percentage of occurrence of events, and "mean, standard deviation" of values. If required, certain cases were tested to confirm diagnosis of autoimmune disease.

Statistical Analysis. All analyses were performed using SPSS, version 18.0 (SPSS Inc., Armonk, NY, USA). The demographic characteristics of cases and controls were compared using the Fisher exact test, adjusted Odds Ratios, and 95% Confidence Intervals (CIs) calculation.

6. RESULTS

This study was designed to investigate the association between CO_2 & Ca^{2+} and autoimmune disorders. The study is limited to CO_2 and Ca^{2+} effects, thus future large studies should evaluate further the impact of vitamin D as well. However, the most important question remains unanswered: whether low vitamin D is simply reflecting greater autoimmune disease severity or represents an independent risk factor. This question is meaningful, since there are many factors contribute to low Vit. D levels and its relation to CO_2 levels.

Our findings derived from matched data, indicated that there is strong association between CO_2 rise and increased autoimmune disorders. Table 1 displays case categorization of participants included in the study. Notably, they are screened during selection to exclude underlying cardiovascular and pulmonary conditions. Table 2 shows demographic characteristics and examination parameters of cases and controls. Table 3 analyzed $PaCO_2$ in cases and controls. To illustrate this finding more clearly, we tracked $PaCO_2$ analysis to test data through two methods of statistical significance.
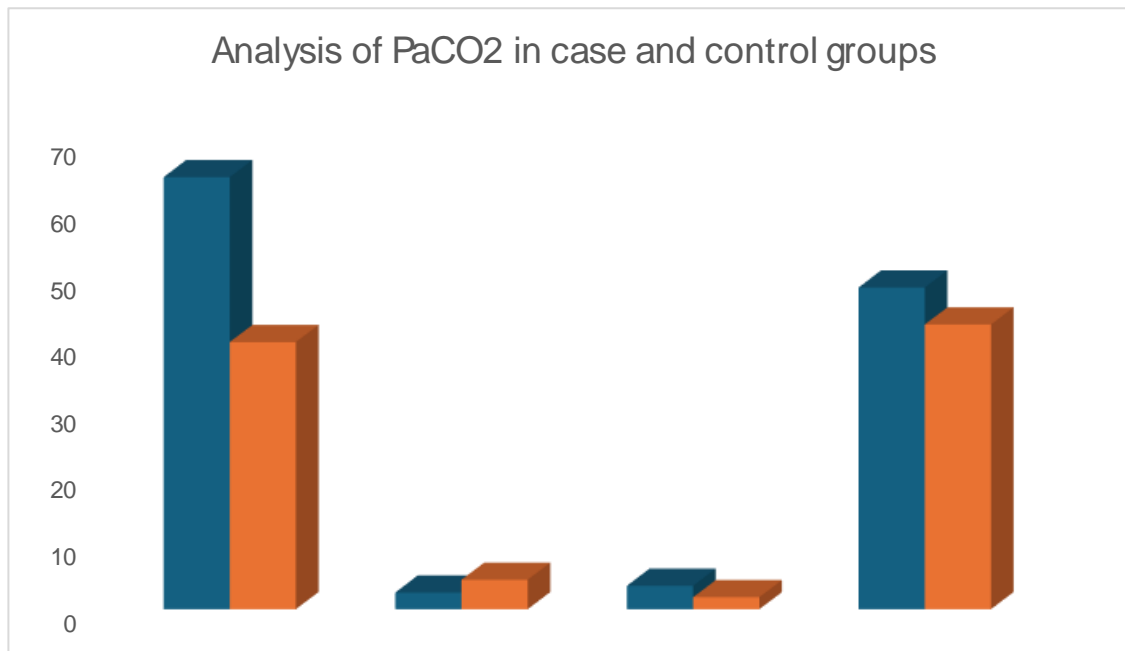
Figure 1: Illustrates the $PaCO_2$ analysis in case and control groups.

Blue colored columns represent patients, while brown colored columns represent controls.

The right category represents mean values of $PaCO_2$, while the left category represents percentage values of increased $PaCO_2$.

Table 1 displays the case categorization of the participants included in the study. Notably, they are screened during selection to exclude underlying cardiovascular and pulmonary conditions that may predispose to increased CO_2 levels.

Table 1: Distribution of diseases among cases.

Case category	Number of cases, n (%)	Male / Female, (60/90)
R A	40 (26.7)	18 / 22
SLE	30 (20)	5/25
JDM	24 (16)	11/13
IBD	18 (12)	9 / 9
Celiac	14 (9.3)	6 / 8
Vitiligo	9 (6)	5 / 4
Psoriasis	9 (6)	4 / 5
EGACU	6 (4)	2 / 4

RA= Rheumatoid Arthritis, SLE= Systemic Lupus Erythematosus, JDM= Juvenile Diabetes Mellitus, IBD= Inflammatory Bowel Diseases, EGACU= Extra Gastric Autoimmune Chronic Urticaria.

Table 2: Demographic characteristics and examination parameters of cases and controls.

Variable	Cases No. (%) (n =150),	Controls No. (%) (n =75),	Odds ratio (95% CI), P value
Sex (male)	60 (40)	32(42.7)	0.8958- (0.511, 1.572), P = 0.3507
Age, mean (S.D.), years	47.9 (14.5)	50.1 (13.4)	(-1.742 to 6.142), SE= 2.00, P= 0.273.
BMI, mean (S.D.), kg/m^2	27.6 (5.1)	26.1 (5.3)	(-2.9401 to -0.0599), SE= 0.73, P= 0.0413
Total annual outcome, $			
- ≤ 12000.	87 (58)	44 (58.7)	
-12000	47 (31.3)	24 (32)	
30000	16 (10.7)	7 (9.3)	
≥ 30000			1.539 (0.744, 3.181), P= 0.1223
Current smoker	34 (22.7)	12(16)	
Obesity total & Rheumatoid			
Obesity=18 & 9	18 (12)	3(4)	3.27(0.932, 11.486), P=0.032
Overweight =19 & 5	19(12.7)	5(6.7)	2.03 (0.727, 5.670), P= 0.088

Obesity was defined as BMI ≥ 30 kg/m^2, and overweight as BMI >25 and <30 kg/m^2. As depicted in table 2, positive associations of obesity with RA were observed in both sexes and in both seropositive and seronegative disease.

Table 3: Analysis of $PaCO_2$ in case and control groups.

Parameter	Cases	Control	95% CI, SE, P value
Pa CO_2 levels (Mean ± SD)	(48.18 ± 12.10)	(42.63 ± 11.06)	(-8.8288 to -2.2712), SE= 1.66. P = 0.001
No (%) of cases with ↑$PaCO_2$, (RR & OR= 95% CI).	97(64.7)	30 (40)	1.6167 & 2.7453 (1.552 to 4.857), P value =0.0005

Pa CO_2 = Partial Pressure of Carbon Dioxide, SD= Standard Deviation.

RR= Relative Risk, OR= Odds Ratio, CI= Confidence interval.

To illustrate this finding more clearly, we tracked Pa CO_2 analysis to test data through two methods of statistical significance; Percentage of occurrence of events, and mean, standard deviation of the values.

7. DISCUSSION

The world is experiencing a life-altering and extraordinary situation in response to climate change. The unique characteristics of carbon dioxide clearly started from impacting the cell electric potential, acidity and homeostasis to its universal level of impact on global warming. The recent advances in understanding the role played by the Ca^2 signalling pathway in the function of diverse immune cells together with the marked increase of autoimmune disorders stimulated our thinking of some underlying correlation between CO_2, and the development of autoimmune disorders. We provide a mechanistic and model context for the interconnection

among CO_2, Ca^{2+}, calcium channels, 2nd messengers and autoimmune disease. This notion was strengthened by the uncovering of a precise relation between CO_2, and Ca^{2+}, and the discovery that Ca^{2+} signals encode information in frequency, kinetics, amplitude, and spatial extent which mediate informational data and communication pathways at cellular and subcellular compartments including portal gates, receptors, cytokines, interleukins, genes and messenger RNA. We found that CO_2 can induce initiation, stabilization, and maintenance of the autoimmune reaction. Evidently, it is the CO2 which provides a particularly bright illustration of the relationships that may exist between its unique multi action characteristics and the multi opinion bizarre of autoimmune process.

This research highlighted the important role of carbon-dioxide-mediated autoimmune disorders. We provide mechanistic insights to unveil and propose the novel concept: "intracellular binding role" played by unique thermo-acidic CO_2, and unique properties of Ca^{2+} channel signal sparks' intensity. Contemplating a coordinated symmetry between CO_2 and Ca^{2+} to get further insights into cellular response to micro-thermally altered proteins. This emerging concept, although highly versatile, but seems homogenous and acts in concert. The harmonically played mechanisms derive from the unique characteristic of both CO_2 and Ca^{2+}. For instance, CO_2 and Ca^{2+} acted as subcellular 2nd messengers, CO_2 has unique thermo-acidic effect on all cells, likewise calcium channels play a critical role in a variety of physiological functions. The special channels (STIMs) act as sensors not only of decreased Ca^{2+} but also of temperature increases and acidosis. Park YJ, et al. [26] explained that the Orai1 mutations in autoimmunity result in decreased expression of several cytokines: IL-1, IL-4, IL-17, IFNγ and TNF alpha in CD4 and CD8 T lymphocytes [26]. Soboloff J, et al. [17] showed that STIMs target proteins other than Orai channels, including voltage operated channels and plasma membrane Ca^{2+} ATPase and SERCA Ca^{2+} [17]. We applied this approach to study the behaviour of selected molecular protein receptors and channels in physiological context of living cells, with unprecedented detail, in sequence of elements mediating stress-induced autoimmune disorders. In view of these findings and evidence for stress-induced autoimmune disorders, we provide the first evidence linking CO_2 and autoimmune disorders. Unexpectedly, our data revealed that increased PCO_2 may be the etiologic pathogenic mechanism of autoimmune disorders. The mechanism is a complex interplay between direct CO_2 local effect on cell membrane and whole cell homeostasis on one side, and Ca^{2+} homeostasis and signalling pathways on the other side. According to Phelan DE, et al. [28] CO_2 acts as central signalling hub in gene transcription and cytokine regulation. By reemitting infrared, CO_2 instantly causes rapid increase in cell temperature and excitability through electrostatic energy. Ahamad N, et al. [29], indicated that Ca^{2+} entry increases activation of pathways involved nuclear factor" NFκB" translocation into nucleus to activate transcription of gene networks related to cell survival.

While it was previously believed that CO_2 moves across biological membranes only by passive diffusion, we evidently introduce the novel concept of CO_2 "drill-like" action and propose existence of other channels that enable CO_2 to penetrate cell membranes acting as an electric drill, forcing Ca^{2+} to get inside cells against the concentration gradient. This increased intracellular calcium influx augments 2nd messenger signal that CO_2 has generated by its immediate intracellular entry.

At cellular level, Mistrik M, et al. [30] reported that thermal damage primarily impairs proteins, causing their unfolding, aggregation, and denaturation [30]. Nadal E, et al. [31] added that adaptive responses to heat stress depend on intensity of micro-heat damage and involves an extensive reorganization of gene expression [31].

Heating reversibly alters electrical capacitance of plasma membranes, depolarizing the target cells, triggering thermosensitive ion channels, ultimately forming membrane pores, and increasing conductance which activate intracellular second messengers. Tsai S-R, et al. [32] assessed the role of infrared-stimulated and activated transmembrane ion channels which generate selective rechargeable electrolytic bio-battery with pathways including Ca^{2+}, ATP and GTP, that provide energy for cellular reactions including signalling, and gene transcription [32].

Our findings show that CO_2 imposed acidic milieu critical in regulating signalling cascade and Ca^{2+}-dependent transcription factors, which regulate gene activity through three important pathways culminating in an autoimmune commence. CO_2 diffusely penetrated cell membranes and maintained intracellular acidity as well. Thiel G, et al. [33] has detailed these pathways. To respond to changes in microenvironment, cells need to switch genes on and off. One mechanism that links events at cell surface to gene expression in nucleus employs intracellular Ca^{2+}. It seems clear CO_2 effects on immune cell functions are dependent on pH changes and molecular CO_2 sensing, which employ action potential generated ion channels.

Similarly, the mechanism of intracytoplasmic Ca^{2+}-mediated-autoimmune disorders seems pivotal in generating cytokine pathways, and transcription factors which are key determinant in autoimmune disorders together with CO_2. This is achieved through a communication network involving channels, transcription factors, and gene regulation. Thiel G, et al. [33] and Yeh YC, et al. [34] confirmed our proposal [33,34]. Given that CO_2 altering expression of genes of immunity, and Ca^{2+} influx activated NFAT, NF-κB, and c-fos factors which work in tandem to control cytokines, whose outcome is immune tolerance [26,34]. Guo Q, et al. [35] explicated that oscillatory activation of NF-κB promotes transcription of inflammatory genes, whereas persistent activation reprograms epigenome to involve a broader range of genes [35].

In context of experiments examining impact of acidity on immune cell functions, it is important to consider cell type, receptors, and responses. The link established here between acidity stress signalling and immune functioning is consistent with Davern's study, which documented that acidity suppresses anti-tumor T lymphocyte function by a significant upregulation of inhibitory immune checkpoints, CTLA-4 [20]. Navarro, and Sun, reported that addition of ICBs (Immune Checkpoint Blockade) to target ICs (Immune checkpoints) that were upregulated under severe acidity may be necessary to overcome treatment resistance [21,23]. These findings establish that acidosis-induced upregulation of immune checkpoints on T cells may potentially contribute to immune evasion, and upregulation of CTLA-4 which plays a key role in early development of autoreactive T-cells by skewing the balance between destructive T-effector cells and protective Treg cells [23,36]. Taken together and results of Peter et al. that clarified importance of CTLA-4 and PD-1/PD-L1 in protecting heart from autoimmune myocarditis, we conclude that CO_2 can promote autoimmune diseases. This is consistent with many other studies which have demonstrated that CTLA-4 is a critical negative regulator of T cell activation and autoreactivity such as Hossen et al and Guo et al [35-37].

T cells role in immune homeostasis. Precise regulation of T cell activation is crucial for overall immune homeostasis. Microenvironmental cues and signalling pathways are required for T cell activation. In this context, CO_2 can activate signalling pathways and transcriptional alterations leading autoreactive T cells to be in a state of activation and development of autoimmune diseases. This may occur by CO_2 alone and/or combined CO_2 Calcium circuit. The biological pathways most associated with differential gene expression may be compensatory reactions to limit injury from altered inflammatory activity. In line with our study, we conclude

that transcriptional response to elevated CO_2 may alter gene expression. Casalino-Matsuda SM, et al. [38] found that hypercapnia downregulated expression of 183 genes, among these are genes linked to immune responses [38]. To further understand the mechanism, we proposed that acidic medium involves Histidine's protonation resulting in imidazole ring ionization, and conformational changes promoting heterotrimeric G proteins resulting in increased production of intracellular 2nd messengers cAMP [39].

Our study was conducted on autoimmune cases, indicated that CO_2 expressed its effect by multiple mechanisms and diverse actions such as, direct effect, Ca^{2+}-induced effect, and pathways that finally impact overall autoimmune processes. To further understand these mechanisms and pathways we analyzed data from Marchesan E, [40] et al. to prove finding [40].

Significantly, CO_2 provides constant physiological pH, through CO_2-bicarbonate buffer system as it maintains optimum temperature in cell culture [41]. In other words, CO_2 creates suitable conditions for biologic cellular processes including increased intracellular Ca^{2+} with subsequent activation of various transcription factors, receptors, and channels.

In summary, considering the CO_2 role in cell culture, CO_2 not only induced autoimmune process, but also maintained autoimmune disorder culminating in appearance of the disease. This explains why some serologic markers of autoimmune disorder can be identified long time before the disease manifest clinically.

CO_2 impacted autoimmune antigen-antibody" Ag-Ab" reaction, Epitope-paratope. Our study investigated CO_2 chemical effect on autoimmune interaction. The CO_2 chemo-reflex is required for tonic drive underpinning electrostatic forces and paratope-epitope bonding. The chemical autoimmunity induced by chemical bonds is catalysed by CO_2 rise; In hope of gaining a better understanding of how CO_2 impacted autoimmune reaction, we analyzed data from Quantum mechanics to understand another aspect of autoimmune reaction. It described; all chemical bonds are based on electrostatic forces. Ag-Ab reactions are stabilized at low temperature, as high temperature modulates their binding kinetics, diffusivity, and aggregation propensity. So, to dissociate antigen–antibody complexes formed, one would have to raise temperature to $56^{°C}$ [42]. B-cells can neutralize pathogenic molecules by specifically targeting them using receptors on their surfaces. This is achieved *via* molecular interactions between paratope and epitope [43]. Antigens are molecular structures found on the surface of pathogens (bacteria, viruses, and other foreign substances), as well as on the surface of body cells. Antibodies or immunoglobulins are formed by "B-cells" in response to antigens. Binding of Ag-Ab reaction relies on specific interaction of amino acids at paratope-epitope interface. Akbar R, et al. [44]. reported that a fundamental premise for predictability of antibody-antigen binding is existence of paratope-epitope interaction motifs that are universally shared among antigen-antibody structures. Biochemical reaction between antibodies and specific antigens occurs when come closer to nanometres as they react in a 'lock-and-key' manner. When combination between lock and key is precise (in terms of geometry and chemical character), the goodness of fit is high, and the reaction will be stronger. The strength of bond between antigen and antibody known as "antibody affinity" depends on non-covalent bonds such as hydrogen bonds, and electrostatic forces. Predictors of antigen-binding affinity are critical for therapeutic interventions of antibodies since binding affinity between antibody and epitope occurs only if their structures are complementary [42,45]. Goodness of fit relies on time. Too short time means that antigen and antibody may not have had sufficient time to form a good reaction, prolonged time causes antigen–antibody complexes to dissociate. Other fitness factors include temperature, pH, and ionic strength. Low ionic strength solutions are commonly used to increase sensitivity of Ag-Ab reactions [42,46].

These observations support that local acidity could influence bioactivity and distribution of antibodies by weakening electrostatic interactions and/or hydrogen bonds, interfering with clinical efficacy of antibodies. Extreme p^H values induce marked conformational changes in antibody molecule that probably destroy complementarity with antigen [46]. Moreover, decreased binding of antibody to antigen at acidic endosomal pH results in reduced recycling of antibody/antigen complexes and increased lysosomal trafficking of antigen for degradation, thus decreasing targeted antigen serum level [47]. Hironiwa confirmed that Ca^{2+} dependent antigen-binding antibody can dissociate its antigen in endosome and accelerate antigen clearance [48].

In summary, aside from factors that negatively influence affinity and efficacy of the antigen-antibody reaction, CO_2 has been identified as ideal substance to inhibit antigen-antibody reactions at site of inflammation forcing antibodies to leave to nearby location away from local CO_2 effect. Once antibodies found appropriate conditions for reaction, they become instantly available to interact with self-antigens and launching autoimmune process that depends on molecular complementarity between self-antigen and antibody. Low ionic strength in the new site (away from CO_2) enhances self Ag-Ab reaction and increasing antigen-antibody association. Based on microenvironment enhanced autoimmune process, we speculate that specific Ag-Ab reaction is a pattern of biochemical reaction defined by cellular CO_2 sensitivity and mediated by p^H changes and other chemical factors. Our work identified the first evidence linking CO_2 influence on epitope-paratope reaction, broadly analogous with physicochemical features of antigen-antibody autoimmune responses. We concluded that information contained within antibody is potentially used to gain insight into physicochemical properties of the cognate epitope. In accord with this view, Boswell CA, et al. [49] demonstrated a high correlation between corresponding structural properties of paratope and epitope [49]. Stank A, et al. [50] found that orientation of a single amino acid side chain in substrate binding pocket of the active site caused increased affinity [50]. Because an epitope corresponds to a small region (surface area of 4-6 amino acids), it is possible for different macromolecules to exhibit the same molecular identities and orientations. Antibodies secreted after binding to one epitope on an antigen may exhibit cross reactivity for similar epitopes on different antigens. Cross reactivity occurs when an antibody binds not to the antigen that elicited its synthesis and secretion, but to a different antigen such as that of nearby host antigens with nearly similar epitopes [51]. Thus, impelling antigen antibody reaction to be confined to host cells, culminating in established autoimmunity. This progressing autoimmune process is magnified by antibody kinetics which govern their binding to antigens and other cognate receptors. It is evidenced that electrostatic interactions between anionic cell membranes and positive surface charge of antibodies can influence tissue disposition kinetics in a manner independent of antigen recognition [49].

It is evident that CO_2 thermic effect can increase mobile functional antibodies to move away from antigens that elicited their synthesis, then generalized protonation of CO_2 increases electrostatic interactions between anionic cell membranes and positive surface charge of antibodies to be oriented to host antigens instead of antigens elicited their synthesis, thus initiating and boosting Ag-Ab autoimmune reaction. This notion was demonstrated from Reverberi, who described Ag-Ab reaction and hydrogen bonds are exothermic, and heat derives from energy released through a chemical bond formation. As temperature is a critical determinant in many physicochemical, and biological processes, such as diffusion, reaction equilibrium and kinetics, the initial high temperature can increase proportion of mobile functional antibodies [45,46]. According to these thermodynamic principles, we can explore CO_2 thermic effect which catalyses initiating autoimmune reaction, then after the

reaction had been established, the total exothermic effects of both reaction and hydrogen bond cause heat energy release which speeds more antibodies to bind more host antigens. By time, autoimmune process would become organized and boosted by hypercapnic acidosis which impairs ability of lymphocytes to distinguish between self and non-self. This accords with Almanza [52].

In summary, CO_2 catalysed initiating, maintaining, and providing an optimum temperature and pH, mediated by receptors, proteins and signalling pathways.

Our results suggest that high CO_2 is involved in autoimmune damage of certain tissues such as cartilage, and ligaments in certain cases (RA, SLE, diabetes), gastrointestinal tract (IBS, Celiac), and skin (Vitiligo, Urticaria). We suggest the reason behind selective damage of these tissues is due to electrostatic interactions between positive surface charge of antibodies and anionic cell membranes of negatively charged tissues in a manner that is independent of antigen recognition. Our suggestion may align with Veda et al, and Leal et al, who stated that human body contains a multitude of negatively charged tissues including skin, joint, cartilage and ligaments, mucosa of GIT, and vitreous of eye, [53,54]. Furthermore, Nadal et al, evidenced that pattern of gene expression in response to heat stress is affected by fine regulation of mRNA synthesis with diverse phenotypic expression [31]. Mistrik M, et al. [30] discussed the emerging field of plasmonic Nanoparticles (NPs) where absorption of light by NPs may affect plasmon resonance which depends on many parameters, including size, shape, and dielectric properties of NPs culminating in varied heat expression effects [30].

Carbon dioxide effect on immune cells and signal transduction. Different determinants have been reported to influence magnitude of autoimmune response, including inflammatory cytokines, costimulatory signals, second messengers and microenvironment. The interplay between (CO_2, Ca^{2+}) and other determinants governs the outcome of reaction. Studies found that increased CO_2 pressure reduces pH, which increases extracellular adenosine concentration [55]. These signalling events contribute to a range of physiological responses, some of which are sustained and involve CO_2-dependent suppression of NF-κ B-dependent immune regulatory cytokines involved in antibody class switching and immunological memory formation [28].

Antigen presentation & T cells. A critical element of immune function is the efficient interaction between activated APCs and T cells. Antigen presentation by "MHC" on APCs caused T Cell Receptor (TCR) internalization and gene rearrangement at endogenous TCRα locus [56]. A key question, which remains to be answered with respect to the MHC-TCR immune complexes binding, and selective orientation to host antigens instead of anti-microbial complexes binding. Obviously, change in ambient conditions caused by increased CO_2 is reflected on binding topologies of TCR-MHC and the observed differences between autoimmune complexes and anti-microbial complexes as demonstrated by Wucherpfennig KW, et al. [57]. It is the CO_2 thermo-acidic effect that creates curvature and fluidity of immune cell membranes with induction of topographic changes and mobilization of the membrane-linked molecules including TCR-MHC immune complexes.

Effect of CO_2 on other immune cells. Inflammation negatively regulates Memory Precursor Effector Cell development (MPEC). Notably, we are aware that elevated CO_2 generates anti-inflammatory effects *via* acidification. Elevated CO_2 correlates with reduced monocyte and macrophage migration and inflammatory gene expression [58]. Several types of inflammatory molecules including IL-12, IFNγ, are known to inhibit acquisition of memory [59].

Antibody diversity and B cell. It is recognized that autophagy plays a key role in cell-type specific immune cell development and differentiation. Our study revealed the role of CO_2 in driving B cell autoimmunity. This role is

concluded from Soboloff J, et al. [17] and Raza IGA, [60] et al. By acting as sensors of temperature increases and acidosis, Ca^{2+} Channels STIM1/Orai1 can increase autophagy. Autophagy supports self-reactive B cells in subverting autoimmune checkpoints to present autoantigens to T-lymphocytes [17,60]. Additionally, autophagy enables B cells to present peptides derived from self–antigens to cognate T cells. So, CO_2 can promote B cell autoimmunity through its acid-thermic effects on Ca^{2+} Channel STIM1/Orai1- autophagy pathway.

Role of antibody errors during random recombination of gene segments has been expertly reviewed. These errors are one of the sources of antibody diversity. B cells have unique property to somatically alter their immunoglobulin genes by recombination, hypermutation and class-switch recombination. Their mutations are initiated by Activation-Induced Cytidine Deaminase (AID), which deaminates cytosine [61].

Our results further attest to biological significance of a multifaceted immune-cellular response to thermal damage. Several studies yielded supporting findings to our results.; Shiraz AK, et al. [62] demonstrated that somatically mutated high-affinity autoantibodies are a hallmark of autoimmune diseases [62]. Jaiswal A, et al. [61] confirmed that somatic hypermutation introduces point mutations into immunoglobulin genes and initiated by cytosine deamination and completed by error-prone processing of resulting uracil by base excision repair factors [61]. Ehrlich M, et al. [63] found that DNA cytosine methylation and deamination were accelerated by heat [63]. Moreover, Sethi reported that acceleration of cytosine deamination is due to other factors such as protonation of free phosphate groups of cytosine [64]. Mikocziova I, et al. [65] demonstrated that certain proteins can modulate B cell responses and MHC genes, [65]. Consequently, CO_2 by its thermal and protonation abilities could thereby affect ability of B cells to respond to antigens with production of somatically mutated high-affinity autoantibodies through thermal effect of "CO_2" on cytosine deamination. Also, CO_2 protonation can dissociate phosphate groups to help attack cytosine amino group. However, Blake et al attributed altered protein function to carbamate post-translational modification, mediated by the attack of CO_2 on N-terminal α-amino groups and it is considered part of CO2 sensing and signalling mechanism [66]. Furthermore, Casalino et al, found that CO_2 induction of mutated antibodies may be evidenced through hypercapnic downregulation of major genes linked to immune responses [38]. Interestingly, in line with our findings, Zhao et al, found that CO_2 can suppress catalytic antibody activated carbamate. Catalytic antibodies made it feasible to prevent emergence of autoimmunity [67]. Collectively, these data support our conclusion that high CO_2 promotes emergence of autoimmune disorders in a pH-dependent manner.

In summary, multiple sites within the immune system can elicit adaptive responses to local CO_2 concentrations. The evidence points to strong contribution of acid/p^H CO_2 sensing to modulate immune response. In this context, there is ample evidence that CO_2 exerts changes on catalytic antibodies towards benefit of autoimmunity.

CO2, T-cell activation, and function. Autoimmune induction by thermo-acidic effects unique to CO_2, influences both TCR and BCR integrity and function of T and B cells respectively. The mechanism includes modulation of cellular cholesterol, and topological variations between TCRs and other membrane proteins which induce membrane bending to micro adhesion rings. CO_2 modulates cellular cholesterol accumulation, and upregulation of lipid metabolism genes [68]. Robinson GA, et al. [69] showed that cholesterol enriched in plasma membrane forms signalling platforms called lipid rafts, essential for T-cell activation and function. So, targeting lipid metabolism in T-cells is a promising autoimmune treatment [69]. TCR signal is initiated by phosphorylation of

motifs (ITAMs) contained within cytoplasmic domains of the invariant subunits; ITAMs are critical to initiate signalling following ligand engagement [70]. Unexpectedly, Gaud et al, found that T cells expressing TCRs containing inactivated CD3ζ ITAMs, exhibited reduced ability to discriminate between low- and high-affinity ligands, resulting in enhanced signalling and cytokine responses to low-affinity ligands. Also, TCRs were refractory to antagonism. Finally, T cells expressing 6F-CD3ζ displayed enhanced cytolytic activity against solid tumors expressing low-affinity ligands, identifying a new counterintuitive approach to TCR-mediated cancer immunotherapy [71].

Our work provides new evidence of dramatic change of subcellular surface receptor topology proteins in response to mechanical forces and thermally induced membrane fluctuation, which mediate tolerance to immune activation in response to heat. Al-Aghbar MA, et al. [72], found that surface receptor topology can exert mechanical forces to induce curvature around engaged p MHC/TCR complexes to release CD3 which induced tolerance to immune activation [72]. It was seen that the rapid influx of Ca^{2+} may compete with negatively charged phospholipids, thus releasing CD3 ITAMs from plasma membrane [73].

In summary, CO_2 modulates MHC/TCR complexes between the T cell-APC interface through; 1st. Thermally induced mechanically forced membrane fluctuation induced curvature around engaged p MHC/TCR complexes which releases CD3 cytoplasmic domains. Second, CO_2 protonation of negatively charged phospholipids released CD3 ITAMs from membranes. The overall outcome may affect ligand affinity and discriminative ability between self and non-self-antigens. 3rd, CO2 thermal effect is linked to cellular stress signalling pathways, like heat shock proteins, with expected role in ribosomal DNA gene translation to protein due to subcellular mobilization [74]. 4th, acidic microenvironment has direct effect on shaping T-cell biology through affecting Ca^{2+} responses to TCR stimulation for acquisition of T cell full functional competence to avoid autoimmunity. The outcome would blunt immunity. 5th, Yang P, et al [75] discussed that low pH inhibits IFN$^\gamma$ → upregulated Th17 cells which are involved in pathogenesis of diverse autoimmune diseases.

In conclusion, CO_2 plays a major role in orchestrating the immunologic response after a major insult of disturbed Ca^{2+} homeostasis.

Other studies have yielded contradictory findings to our conclusion. A meta-analysis stated that Methylenetetrahydrofolate reductase polymorphisms (MTHFR 677 C/T) were risk factor for autoimmunity [76]. The small number of studies included and heterogeneity among studies did affect their results. Sample size is still moderate, adding weak evidence of "MTHFR" effect on autoimmunity.

Our results explored surprising association between H. pylori infection and extra gastric autoimmune, chronic urticaria. We suggest that urease enzyme produced NH_3 and CO_2 from urea. Gradual build-up of CO_2 develops extra gastric autoimmune diseases, including urticaria as evidenced by studies [77] which described that H. pylori elicit numerous adaptive mechanisms. Urease seems to be the most virulence factor essential for H. pylori colonization and survival.

Our study has several methodological strengths. 1st, study groups were obtained from hospitals, sample size was relatively large in terms of case control measures, and data results are reflective of target population, with a minimal likelihood of loss to follow-up cases, and recall bias. 2nd, we employed demographic matching of cases and controls to minimize potential confounders. 3rd, personal bias could not apply to blood work.

The CO_2-induced gastrointestinal manifestations can be explained considering Abdelrazak' studies, who discovered; H. pylori caused infantile colic, and demonstrated that H pylori LPS–activated TLR4 releases IL-8

from gastric epithelia, initiating inflammatory damage. The interaction between host immune factors and H. pylori virulence factors determines the outcome of H. pylori infection [77]. Furthermore, this course induces Ca^2 influx which prolongs IL-8-induced CO_2 signals [78].

Our results contradict molecular mimicry. Studies have conflicting opinions as large number of infectious agents may prevent autoimmune disease. Hygiene hypothesis theory breaches integrity of molecular mimicry [79]. Evidently, we propose that molecular mimicry is not a pathogenic mechanism of autoimmune diseases, rather, the specific inflammatory mediators associated with increased expression of autoreactivity. For instance, Komastu T, et al. [80] showed that Th1 CD4+ cells induced IL-12, are critical factors in viral immunity [80]. Callahan et al, presented that during Campylobacter infection, dendritic cells and macrophages present processed *C. jejuni* antigens to T lymphocytes, which develop into Th1, Th17 CD4+ T lymphocytes, with secretion of IFN-γ and IL-17 [81], which are characterized by promoting post-inflammatory autoimmune Guillan-Barre syndrome. Furthermore, Soderholm et al, reported that the Th1 and Th17 responses play significant roles in adaptive immunity to Group A streptococcus pharyngitis [82], which causes rheumatic fever and rheumatic chorea.

8. CONCLUSION

The implications of this study are far-reaching, as it opens avenue of research in the pathogenesis, treatment, and prevention of autoimmune disorders. With the rapid accumulation of immunological knowledge, it will predict that improvement in the issues of selectivity, efficacy, and pharmacokinetics of the immune modulator drugs. We found that CO_2 can induce initiation, stabilization, and maintenance of the autoimmune reaction.

- CO_2 penetrates cell membranes to act as an electric drill with the result of forcing Ca^2 to get inside the cells against their concentration gradient "CO_2 drill like action". This increased intracellular calcium influx augments the second messenger CO_2 signal that just has made by its immediate intracellular entry. The CO_2 induced heating reversibly alters the electrical capacitance of the plasma membrane, depolarizing the target cell to affect ion channel gating, and triggering thermosensitive ion channels forming membrane pores.

- The mechanism of autoimmune induction by both thermal and protonation effects unique to CO_2, was shown to influence both TCR and BCR integrity and function of T and B cells respectively. It is caused via cellular cholesterol modulation and topological variations between TCRs and other membrane proteins which induce membrane bending and micro adhesion rings.

- Somatically mutated high-affinity autoantibodies are produced through the CO_2 thermal cytosine deamination and protonation, to dissociate phosphate groups which attack cytosine amino group. On the other hand, the CO_2 induction of mutated antibodies may be further catalysed by the "MHC" susceptibility genes for autoimmune diseases.

9. FUTURE PROSPECTIVES

- Continued investigation of the role of CO_2 in the pathogenesis of various autoimmune diseases, should represent the most important intent in future studies. The precise determination of genes involved in

immune mediated CO_2 pathways along with prediction of possible mutations and their effects on immune response must be considered.

- The pivotal role of CO_2 in eliciting immune response makes this identification more essential to increase understanding of mechanisms underlying the affinity of autoantibodies to self-antigens and hence improving the design of therapeutic monoclonal antibodies. Hopefully, the evidence presented herein linking CO_2 to the pathogenesis of autoimmune diseases will encourage investigators of clinical trials to find new methods to overcome the undesired issues of antibodies against urease. The aim should aid in designing future studies of urease inhibitors to develop efficient new generations with the least possible side effects. It will uncover new avenue to eradicate H pylori and its dangerous complications. In addition, it will abort the development of autoimmune disorders because demolishing urease can stop sustained CO_2 build-up, thus prevent autoimmune process, and make H pylori survival unlikely. Pending such studies, debate will continue regarding potential benefits and harms of hypercapnia, and whether therapeutic hypocapnia or normocapnia holds the best promise.

- Attention should be paid to a new line of therapies that include modalities to deplete CO_2 rise and to compete with its intracellular diffusion by employing hyperbaric oxygen to neutralize CO_2 deleterious effects. Overall, we present a versatile modality that may be broadly applicable in pharmaceutical industries, including diverse strategic mechanisms to search for immunologic modulators of both autoimmune disorders and cancer.

- To address the issue of various autoimmune mediated Ca^{2+} channels and their precise targeted drug delivery, we must ensure the specificity of developed drugs for the Ca^{2+} channels to reduce cross-reactivity and damage to other tissues. We should be aware of the mechanisms by which drugs can more effectively modulate a specific Ca^{2+} channel.

- Our study raises multiple questions that should inspire further research dedicated to the processing of cellular channels, pathways and altered molecular receptor proteins, to target their consequent damaging effects.

REFERENCES

1. Marin TW, Janik T. Ultraviolet spectroscopy of pressurized and supercritical carbon dioxide. Commun Chem. 2021;4(1):77.

2. Geers C, Gros G. Carbon Dioxide Transport and Carbonic Anhydrase in Blood and Muscle. Physiol Rev. 2000;80(2):681-715.

3. Patel S, Jose A, Mohiuddin SS. Physiology, Oxygen Transport And Carbon Dioxide Dissociation Curve. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan. 2023 Mar 27. Available from: https://www.ncbi.nlm.nih.gov/books/NBK539815/

4. Almanza-Hurtado A, Polanco Guerra C, Martínez-Ávila MC, Borré-Naranjo D, Rodríguez-Yanez T, Dueñas-Castell C. Hypercapnia from Physiology to Practice. Int J Clin Pract. 2022;2022:2635616.

5. Shapiro M, Homma K, Villarreal S, Richter C-P, Bezanilla F. Infrared light excites cells by changing their electrical capacitance. Nat Commun. 2012;3:736.

6. Garcia AJ, Ramirez JM. Keeping carbon dioxide in check. Elife. 2017;6:e27563.

7. Wen X, Li B. A population-based study on autoimmune disease. Lancet. 2023;401(10391):1829-31.

8. Rosenblum MD, Remedios KA, Abbas AK. Mechanisms of human autoimmunity. J Clin Invest. 2015;125(6):2228-33.

9. Rojas M, Herrán M, Ramírez-Santana C, Leung PSC, Anaya JM, Ridgway WM, et al. Molecular mimicry and autoimmunity in the time of COVID-19. J Autoimmun. 2023;139:103070.

10. Pisetsky DS. Pathogenesis of autoimmune disease. Nat Rev Nephrol. 2023;19(8):509-4.

11. Lee KH, Ahn BS, Cha D, Jang WW, Choi E, Park S, et al. Understanding the immunopathogenesis of autoimmune diseases by animal studies using gene modulation: A comprehensive review. Autoimmun Rev. 2020;19(3):102469.

12. Oh B-C. Phosphoinositide's and intracellular calcium signaling novel insights into phosphoinositide's and calcium coupling as negative regulators of cellular signaling. Exp Mol Med. 2023;55(8):1702-12.

13. Moccia F, Fiorio Pla A, Lim D, Lodola F, Gerbino A. Intracellular Ca^{2+} signaling unexpected new roles for the usual suspect. Front Physiol. 2023;14:1210085.

14. Bootman MD, Bultynck G. Fundamentals of Cellular Calcium Signaling: A Primer. Cold Spring Harb Perspect. Biol. 2020;12(1):a038802.

15. Bkaily G, Jacques D. Calcium Homeostasis, Transporters, and Blockers in Health and Diseases of the Cardiovascular System. Int J Mol Sci. 2023;24(10):8803.

16. Fahrner M, Grabmayr H, Romanin C. Mechanism of STIM activation. Curr Opin Physiol. 2020;74-9.

17. Soboloff J, Rothberg B, Madesh M, Gill DL. STIM proteins: dynamic calcium signal transducers. Nat Rev Mol Cell Biol. 2012;13(9):549-65.

18. Trebak M, Kinet JP. Calcium signaling in T cells. Nat Rev Immunol. 2019;19(3):154-69.

19. Kuo IY, Ehrlich BE. Location, location, and activation of a channel by calcium. Proc Natl Acad. Sci U S A. 2022;119(43):e2214826119.

20. Davern M, Donlon NE, O'Connell F, Gaughan C, O'Donovan C, Habash M, et al. Acidosis significantly alters immune checkpoint expression profiles of T cells from esophageal adenocarcinoma patients. Cancer Immunol Immunother. 2023;72(1):55-71.

21. Navarro F, Casares N, Martín-Otal C, Lasarte-Cía L, Gorraiz M, Sarrión P, et al. Overcoming T cell dysfunction in acidic pH to enhance adaptive T cell transfer immunotherapy. Oncoimmunology. 2022;11(1):2070337.

22. Meng L, Wu H, Wu J, Ding P, He J, Sang M, et al. Mechanisms of immune checkpoint inhibitors: insights into the regulation of circular RNAS involved in cancer hallmarks. Cell Death Dis. 2024;15(1):3.

23. Sun Q, Hong Z, Zhang C, Wang L, Han Z, Ma Q. Immune checkpoint therapy for solid tumours: clinical dilemmas and future trends. Signal Transduct Target Ther. 2023;8(1):320.

24. Tai X, Van Laethem F, Sharpe AH, Singer A. Induction of autoimmune disease in CTLA-4-/- mice depends on a specific CD28 motif that is required for in vivo co stimulation. Proc Natl Acad Sci U S A. 2007;104(34):13756-61.

25. Izquierdo J-H, Bonilla-Abadía F, Cañas CA, Tobón GJ. Calcium Channels, Intracellular Signaling and Autoimmunity. Reumatol Clin. 2014;10(1):43-7.

26. Park YJ, Yoo SA, Kim M, Kim W-U. The Role of Calcium-Calcineurin-NFAT Signaling Pathway in Health and Autoimmune Diseases. Front Immunol. 2020;11:195.

27. Watad A, Azrielant S, Bragazzi NL, Sharif K, David P, Katz I, et al. Seasonality and autoimmune diseases: The contribution of the four seasons to the mosaic of autoimmunity. J Autoimmun. 2017:82:13-30.

28. Phelan DE, Mota C, Lai C, Kierans SJ, Cummins CP. Carbon dioxide-dependent signal transduction in mammalian systems. Interface Focus. 2021;11(2):20200033.

29. Ahamad N, Sun Y, Da Conceicao VN, R D Xavier Paul Ezhilan C, Natarajan M, Singh BB. Differential activation of Ca2+ influx channels modulate stem cell potency, their proliferation/viability and tissue regeneration. NPJ Regen Med. 2021;6(1):67.

30. Mistrik M, Skrott Z, Muller P, Panacek A, Hochvaldova L, Chroma K, et al. Microthermal-induced subcellular-targeted protein damage in cells on plasmonic nanosilver-modified surfaces evokes a two-phase HSP-p97/VCP response. Nat Commun. 2021;12:713.

31. de Nadal E, Ammerer G, Posas F. Controlling gene expression in response to stress. Nat Rev Genet. 2011;12(12):833-45.

32. Tsai S-R, Hamblin MR. Biological effects and medical applications of infrared radiation. J Photochem Photobiol B. 2017;170:197-207.

33. Thiel G, Schmidt T, Rössler OG. Ca^{2+} Microdomains, Calcineurin and the Regulation of Gene Transcription. Cells. 2021;10(4):875.

34. Yeh YC, Parekh AB. CRAC Channels and Ca2+-Dependent Gene Expression. In: Kozak JA, Putney JW Jr., editors. Calcium Entry Channels in Non-Excitable Cells. Boca Raton (FL): CRC Press/Taylor & Francis; 2018.

35. Guo Q, Jin Y, Chen X, Ye X, Shen X, Lin M, et al. NF-κB in biology and targeted therapy: new insights and translational implications. Signal Transduct Target Ther. 2024;9(1):53.

36. Hossen MM, Ma Y, Yin Z, Xia Z, Du J, Yi Huang J, et al. Current understanding of CTLA-4: from mechanism to autoimmune diseases. Front Immunol. 2023;14:1198365.

37. Libby P, Lichtman AH. Immunologic Mechanisms of Atherosclerosis and Myocarditis. In: Clinical Immunology 6th ed. 2023.

38. Casalino-Matsuda SM, Wang N, Ruhoff PT, Matsuda H, Nlend MC, Nair A, et al. Hypercapnia alters expression of immune response, nucleosome assembly and lipid metabolism genes in differentiated human bronchial epithelial cells. Sci Rep. 2018;8(1):13508.

39. Winiewska-Szajewska M, Paprocki D, Marzec E, Poznański J. Effect of histidine protonation state on ligand binding at the ATP-binding site of human protein kinase CK2. Sci Rep. 2024;14(1):1463.

40. Marchesan E, Nardin A, Mauri S, Bernardo G, Chander V, Di Paola S, et al. Activation of Ca^{2+} phosphatase Calcineurin regulates Parkin translocation to mitochondria and mitophagy in flies. Cell Death Differ. 2024;31(2):217-38.

41. Klein SG, Alsolami SM, Arossa S. Ramos-Mandujano G, Parry AJ, Steckbauer A, et al. In situ monitoring reveals cellular environmental instabilities in human pluripotent stem cell culture. Commun Biol. 2022;5(1):119.

42. Armstrong B. Antigen-antibody reactions. VOXS. 2020;15:68-80.

43. Jespersen MC, Mahajan S, Peters B, Nielsen M, Marcatili P. Antibody Specific B-Cell Epitope Predictions: Leveraging Information from Antibody-Antigen Protein Complexes. Front Immunol. 2019;10:298.

44. Akbar R, Robert PA, Pavlović M, Jeliazkov JR, Snapkov I, Slabodkin A, et al. A compact vocabulary of paratope-epitope interactions enables predictability of antibody-antigen binding. Cell Rep. 2021;34(11):108856.

45. Reverberi R, Reverberi L. Factors affecting the antigen-antibody reaction. Blood Transfus. 2007;5(4):227-40.

46. Parija SC. Antigen–Antibody Reactions. In Textbook of Microbiology and Immunology. Springer: Singapore; 2023.

47. Devanaboyina SC, Lynch SM, Ober RJ. The effect of pH dependence of antibody-antigen interactions on subcellular trafficking dynamics. MAbs. 2013;5(6):851-9.

48. Hironiwa N, Ishii S, Kadono S, Iwayanagi Y, Mimoto F, Habu K, et al. Calcium-dependent antigen binding as a novel modality for antibody recycling by endosomal antigen dissociation. MAbs. 2016;8(1):65-73.

49. Boswell CA, Tesar DB, Mukhyala K, Theil F-P, Fielder PJ, Khawli LA. Effects of charge on antibody tissue distribution and pharmacokinetics. Bioconjug Chem. 2010;21(12):2153-63.

50. Stank A, Kokh DB, Fuller JC, Wade RC. Protein Binding Pocket Dynamics. Acc Chem Res. 2016;49(5):809-15.

51. Wang SW, Ko YA, Chen CY, Liao KS, Chang YH, Lee HY, et al. Mechanism of Antigen Presentation and Specificity of Antibody Cross-Reactivity Elicited by an Oligosaccharide-Conjugate Cancer Vaccine. J Am Chem Soc. 2023;145(17):9840-49.

52. Almanza-Hurtado A, Polanco Guerra C, Martínez-Ávila MC, Borré-Naranjo D, Rodríguez-Yanez T, Dueñas-Castell C. Hypercapnia from Physiology to Practice. Int J Clin Pract. 2022;2022:2635616.

53. Vedaghavami A, Zhang C, Bajpayee AG. Overcoming negatively charged tissue barriers: Drug delivery using cationic peptides and proteins. Nano Today. 2020;34:100898.

54. Leal J, Smyth HDC, Ghosh D. Physicochemical properties of mucus and their impact on transmucosal drug delivery. Int J Pharm. 2017;532(1):555-72.

55. Garcia-Gil M, Camici M, Allegrini S, Pesi R, Tozzi MG. Metabolic Aspects of Adenosine Functions in the Brain. Front Pharmacol. 2021;12:672182.

56. McGargill MA, Derbinski JM, Hogquist KA. Receptor editing in developing T cells. Nat Immunol. 2000;1(4):336-41.

57. Wucherpfennig KW, Call MJ, Deng L, Mariuzza R. Structural alterations in peptide-MHC recognition by self-reactive T cell receptors. Curr Opin Immunol. 2009;21(6):590-5.

58. Strowitzki MJ, Nelson R, Garcia MP, Tuffs C, Bleul MB, Fitzsimons S, et al. Carbon Dioxide Sensing by Immune Cells Occurs through Carbonic Anhydrase 2-Dependent Changes in Intracellular pH. J Immunol. 2022;208(10):2363-75.

59. Solouki S, Huang W, Elmore J, Limper C, Huang F, August A. TCR Signal Strength and Antigen Affinity Regulate CD8+ Memory T Cells. J Immunol. 2020;205(5):1217-27.

60. Raza IGA, Clarke AJ. B Cell Metabolism and Autophagy in Autoimmunity. Front Immunol. 2021; 12:681105.

61. Jaiswal, A., Roy, R., Tamrakar, A, Singh AK, Kar P, Kodgire P. Activation-induced cytidine deaminase an antibody diversification enzyme interacts with chromatin modifier UBN1 in B-cells. Sci Rep. 2023;13(1):19615.

62. Shiraz AK, Panther EJ, Reilly CM. Altered Germinal-Center Metabolism in B Cells in Autoimmunity. Metabolites. 2022;12(1):40.

63. Ehrlich M, Norris KF, Wang RY, Kuo KC, Gehrke CW. DNA cytosine methylation and heat-induced deamination. Biosci Rep. 1986;6(4):387-93.

64. Sethi S, Takashima T, Nakamura S, Wan L, Honda N, Fujimoto K. Acceleration of the Deamination of Cytosine through Photo-Crosslinking. Curr Issues Mol Biol. 2023;45(6):4687-700.

65. Mikocziova I, Greiff V, Sollid LM. Immunoglobulin germline gene variation and its impact on human disease. Genes Immun. 2021;22(4):205-17.

66. Blake LI, Cann MJ. Carbon Dioxide and the Carbamate Post-Translational Modification. Front Mol Biosci. 2022;9:825706.

67. Zhao D, Chen J, Hu X, Zhang S. Catalytic Antibodies: Design, Expression, and Their Applications in Medicine. Appl Biochem Biotechnol. 2023;195(2):1514-40.

68. Bolshette N, Ezagouri S, Dandavate V, Karavaeva L, Golik M, Wang H, et al. Carbon dioxide regulates cholesterol levels through SREBP2. PLoS Biol. 2023;21(11):e3002367.

69. Robinson GA, Waddington KE, Pineda-Torra I, Jury EC. Transcriptional Regulation of T-Cell Lipid Metabolism: Implications for Plasma Membrane Lipid Rafts and T-Cell Function. Front Immunol. 2017;8:1636.

70. Horváth Á, Erostyák J, Sz″oke É. Effect of Lipid Raft Disruptors on Cell Membrane Fluidity Studied by Fluorescence Spectroscopy. Int J Mol Sci. 2022;23(22):13729.

71. Gaud G, Achar S, Bourassa FXP, Davies J, Hatzihristidis T, Choi S, et al. CD3ζ ITAMs enable ligand discrimination and antagonism by inhibiting TCR signaling in response to low-affinity peptides. Nat Immunol. 2023;24(12):2121-34.

72. Al-Aghbar MA, Jainarayanan AK, Dustin ML, Roffler SR. The interplay between membrane topology and mechanical forces in regulating T cell receptor activity. Commun Biol. 2022;5(1):40.

73. Shi X, Bi Y, Yang W, Guo X, Jiang Y, Wan C, et al. Ca2+ regulates T-cell receptor activation by modulating the charge property of lipids. Nature. 2013;493(7430):111-5.

74. Chatterjee TK, Fisher RA. Mild Heat and Proteotoxic Stress Promote Unique Subcellular Trafficking and Nucleolar Accumulation of RGS6 and Other RGS Proteins. J Biol Chem. 2003;278(32):30272-82.

75. Yang P, Sun Y, Zhang M, Hu L, Wang X, Luo L, et al. The inhibition of CD4+ T cell proinflammatory response by lactic acid is independent of monocarboxylate transporter 1. Scand J Immunol. 2021;94:439-48.

76. Lu M, Peng K, Song L, Luo L, Liang P, Liang Y. Association between Genetic Polymorphisms in Methylenetetrahydrofolate Reductase and Risk of Autoimmune Diseases: A Systematic Review and Meta-Analysis. Dis Markers. 2022;2022:4568145.

77. Ali AM. Helicobacter pylori and infantile colic. Arch Pediatr Adolesc. Med. 2012;166(7):648-50.

78. Schorr W, Swandulla D, Zeilhofer HU. Mechanisms of IL-8-induced Ca^{2+} signaling in human neutrophil granulocytes. Eur J Immunol. 1999;29(3):897-904.

79. Bach JF. Revisiting the Hygiene Hypothesis in the Context of Autoimmunity. Front Immunol. 2021;11:615192.

80. Komastu T, Ireland DD, Reiss CS. IL-12 and viral infections. Cytokine Growth Factor Rev. 1998;9(3-4):277-85.

81. Callahan SM, Dolislager CG, Johnson JG. The Host Cellular Immune Response to Infection by Campylobacter Spp. and Its Role in Disease. Infect Immun. 2021;89(8):e0011621.

82. Soderholm AT, Barnett TC, Sweet MJ, Walker MJ. Group A streptococcal pharyngitis: Immune responses involved in bacterial clearance and GAS-associated immunopathologies. J Leukoc Biol. 2018;103(2):193-213.