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A study discovered the role of carbon dioxide in the pathogenesis of autoimmune disorders

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Abstract

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

Objective: To determine whether CO₂ is associated with autoimmune disorders.

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

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 CO₂, 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 the autoimmune status. Pa CO₂ analysis performed using two methods of statistical significance to validate data.

Results, Pa CO₂ (Mean ± SD) was (48.18 ± 12.10) in autoimmune cases, compared to (42.63 ± 11.06) in control (p= 0.001). Number (%) of cases with ↑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.

Conclusion. Our study confirmed a correlation between CO₂ and autoimmune disorders. The mechanism is a complex interplay between direct effect of CO₂ on cell membrane, calcium "Ca²⁺" homeostasis and signaling pathways. CO₂ thermic effect increases the mobility of antibodies to move away from the antigens which elicited their secretion, then CO₂ 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 CO₂ thermal effect imposes differences in mRNA gene translation with various phenotypic expression. CO₂ directs pathways providing benefits to autoimmunity. A new autoimmune disorder treatment is proposed. Membrane receptors that absorb heat energy reemitted from CO₂ would produce various oscillations of the electron cloud culminating in various phenotypic expression. Due to our study results, it is expectable that a new treatment of autoimmune disorders will prevail in the future.

Keywords. Cell; Calcium; Autoimmune; CO₂; Ca²⁺

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1. Introduction

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

CO₂ molecule is 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 CO₂ 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 hemoglobin as carbamino compounds [2,3]. Hypercapnia is increased partial pressure of carbon dioxide (Pa CO₂) above 45 mm Hg, there are several mechanisms the body should moderate CO₂ by acid-base buffering system. Retention of CO₂ 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].

1.1. 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 signaling 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].

1.2. Calcium channels and Homeostasis

Cells maintain precise intracellular Ca²⁺ via a complex system of Ca²⁺ channels, transporters, Ca²⁺ ATPases, and signaling effectors, including specific lipid kinases, and phosphatases. Excessive intracellular Ca²⁺ caused functional defects in subcellular organelles such as endoplasmic reticulum "ER", lysosomes, and mitochondria [12].

Calcium is essential for intracellular processes, cell stored Ca²⁺ in high concentrations in (ER) or (SR) which serve as main storage site for calcium. Ca²⁺ channels include all pore-forming, Ca²⁺ permeable proteins [13]. It is demonstrated that Ca²⁺ 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 Ca²⁺ ATPase at ER/SR membrane, called sarcoplasmic/endoplasmic reticulum Ca²⁺-ATPase [15]. When Ca²⁺ stores inside ER/SR becomes depleted, it will be replenished by special calcium channels called store-operated calcium channels, where low Ca²⁺ 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 $\text{CaV}1.2$ 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 signaling 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+} Signaling has been implicated in pathogenesis of autoimmune and congenital immunodeficiency disorders [25]. Dysregulated Ca^{2+} signaling is involved in the pathophysiology of autoimmune diseases [26].

2. Material and Methods

For our knowledge, this is the first novel study to uncover the role of CO_2 in the 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 the 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 diseases which affect CO_2 serum levels such as cardiopulmonary diseases, neuromuscular disorder, critical illness, and multisystem organ failure. Participants were tested for arterial blood gases using ABL90 FLEX PLUS blood gas analyzer 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 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.

2.1. 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.

3. Results

This study was designed to investigate the association between CO₂ & Ca²⁺ and autoimmune disorders. The study is limited to CO₂ and Ca²⁺ 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 CO₂ levels. Our findings derived from matched data, indicated that there is a strong association between CO₂ rise and increased incidence of 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 that may predispose to increased CO₂ levels. Table 2 shows demographic characteristics and examination parameters of cases and controls. Table 3. analyzed PaCO₂ in cases and controls. To illustrate this finding more clearly, we tracked PaCO₂ analysis to test data through two methods of significance, i.e. “percentage of occurrence of events and mean & standard deviation of the values”. Fig.1 illustrates the Pa CO₂ analysis in case and control groups.

Table 1. Distribution of diseases among cases

Case category	Number of cases, n (%)	Male / Female, (60/90)
RA	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 – 30000	47 (31.3)	24 (32)	
- ≥ 30000	16 (10.7)	7 (9.3)	
Current smoker	34 (22.7)	12(16)	1.539 (0.744, 3.181), P= 0.1223
Obesity total & Rheumatoid			
Obesity=18 & 3	18 (12)	3(4)	-0.00468 to 0.16468, P= 0.064
Overweight =19 & 5	19(12.7)	5(6.7)	-0.03053 to 0.15053, P= = 0.194

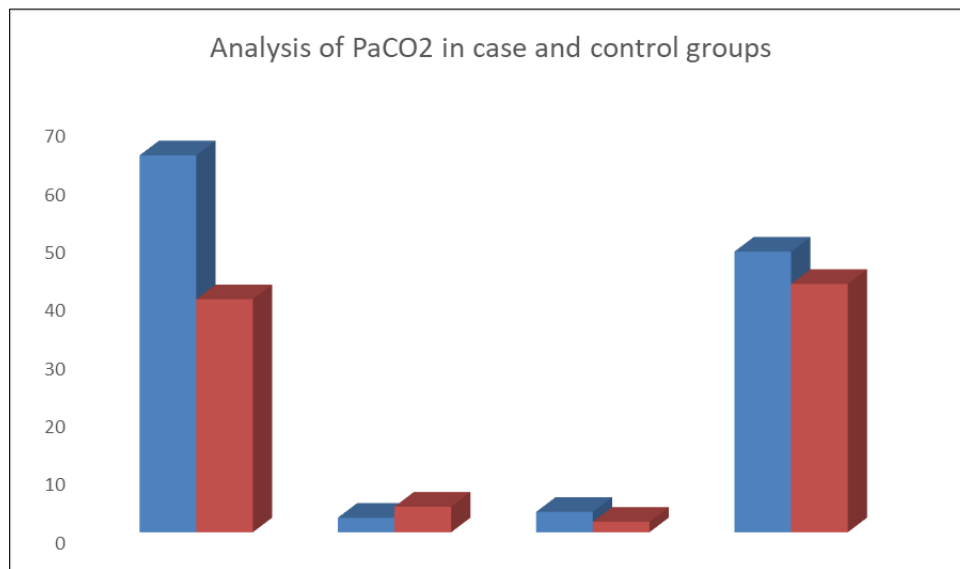
Obesity was defined as BMI ≥ 30 kg/m², and overweight as BMI >25 and <30 kg/m². As depicted in table 2, positive associations of obesity with RA were observed in both sexes and in both seropositive and seronegative disease although it was insignificant.

Table 3 Analysis of PaCO₂ in case and control groups

Parameter	Cases	Control	95% CI, SE, P value
Pa CO ₂ levels (Mean \pm SD)	(48.18 \pm 12.10)	(42.63 \pm 11.06)	(-8.8288 to -2.2712), SE= 1.66. P = 0.001
No (%) of cases with \uparrow 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 CO₂ = 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 CO₂ analysis to test data through two methods of statistical significance; Percentage of occurrence of events, and mean, standard deviation of the values.



Blue coloured columns represent patients, while brown coloured columns represent controls; Right category represents mean values of PaCO₂, while left category represents percentage values of increased PaCO₂.

Figure 1 The Pa CO₂ analysis in case and control groups.

4. 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 Ca²⁺ signaling pathway in the function of diverse immune cells together with the marked increase of autoimmune disorders stimulated our thinking of some underlying correlation between CO₂ and the development of autoimmune disorders. We provide a mechanistic and model context for the interconnection among CO₂, Ca²⁺, calcium channels, 2nd messengers and autoimmune disease. This notion was strengthened by the uncovering of a precise relation between CO₂ and Ca²⁺, and the discovery that Ca²⁺ 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 CO₂ 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 CO₂, and unique properties of Ca²⁺ channel signal sparks and intensity. Contemplating a coordinated symmetry between CO₂ and Ca²⁺ 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 CO₂ and Ca²⁺. For instance, CO₂ and Ca²⁺ acted as subcellular 2nd messengers, CO₂ 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 Ca²⁺ but also of temperature increases and acidosis. Park et al, explained that the Orai1 mutations in autoimmunity result in decreased expression of several cytokines: IL-1, IL-4, IL-17, IFN γ and TNF alpha in CD4 and CD8 T lymphocytes [26]. Soboloff et al, showed that STIMs target proteins other than Orai channels, including voltage operated channels and plasma membrane Ca²⁺ ATPase and SERCA Ca²⁺ [17]. We applied this approach to study the behavior 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 CO₂ and autoimmune disorders. Unexpectedly, our data revealed that increased PCO₂ may be the etiologic pathogenic mechanism of autoimmune disorders. The mechanism is a complex interplay between direct CO₂ local effect on cell membrane and whole cell homeostasis on one side, and Ca²⁺ homeostasis and signaling pathways on the other side. According to Phelan [28], CO₂ acts as central signaling hub in gene transcription and cytokine regulation. By reemitting infrared, CO₂ instantly causes rapid increase in cell temperature and excitability through electrostatic energy. Ahamad et al [29], indicated that Ca²⁺ entry increases activation of pathways involved nuclear factor "NF κ B" translocation into nucleus to activate transcription of gene networks related to cell survival.

While it was previously believed that CO₂ moves across biological membranes only by passive diffusion, we evidently introduce the novel concept of CO₂ "drill-like" action and propose existence of other channels that enable CO₂ to penetrate cell membranes acting as an electric drill, forcing Ca²⁺ to get inside cells against the concentration gradient. This increased intracellular calcium influx augments 2nd messenger signal that CO₂ has generated by its immediate intracellular entry.

At cellular level, Mistrik et al, reported that thermal damage primarily impairs proteins, causing their unfolding, aggregation, and denaturation [30]. Nadal et al, 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 et al, assessed the role of infrared-stimulated and activated transmembrane ion channels which generate selective rechargeable electrolytic bio-battery with pathways including Ca²⁺, ATP and GTP, that provide energy for cellular reactions including signaling, and gene transcription [32].

Our findings show that CO₂ imposed acidic milieu critical in regulating signaling cascade and Ca²⁺-dependent transcription factors, which regulate gene activity through three important pathways culminating in an autoimmune commence. CO₂ diffusely penetrated cell membranes and maintained intracellular acidity as well. Thiel 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 Ca²⁺. It seems clearly that CO₂ effects on immune cell functions are dependent on pH changes and molecular CO₂ sensing, which employ action potential generated ion channels.

Similarly, the mechanism of intracytoplasmic Ca²⁺-mediated-autoimmune disorders seems pivotal in generating cytokine pathways, and transcription factors which are key determinant in autoimmune disorders together with CO₂. This is achieved through a communication network involving channels, transcription factors, and gene regulation. Thiel et al, and Yeh et al, confirmed our proposal [33,34]. Given that CO₂ altering expression of genes of immunity, and Ca²⁺ influx activated NFAT, NF- κ B, and c-fos factors which work in tandem to control cytokines, whose outcome is immune tolerance [26,34]. Guo et al, explicated that oscillatory activation of NF- κ B 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 signaling 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,[37] that clarified importance of CTLA-4 and PD-1/PD-L1 in protecting heart from autoimmune myocarditis, we conclude that CO₂ 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 Guo et al and Hossen et al, [35,36].

4.1. Role of T cells in immune homeostasis

Precise regulation of T cell activation is crucial for overall immune homeostasis. Microenvironmental cues and signaling pathways are required for T cell activation. In this context, CO₂ can activate signaling pathways and transcriptional alterations leading autoreactive T cells to be in a state of activation and development of autoimmune diseases. This may occur by CO₂ alone and/or combined CO₂ 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 CO₂ may alter gene expression. Casalino et al, 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 CO₂ expressed its effect by multiple mechanisms and diverse actions such as, direct effect, Ca²⁺-induced effect, and pathways that finally impact overall autoimmune processes. To further understand these mechanisms and pathways we analyzed data from Marchesan et al, to prove finding [40].

Significantly, CO₂ provides constant physiological pH, through CO₂-bicarbonate buffer system as it maintains optimum temperature in cell culture [41]. In other words, CO₂ creates suitable conditions for biologic cellular processes including increased intracellular Ca²⁺ with subsequent activation of various transcription factors, receptors, and channels.

In summary, considering the CO₂ role in cell culture, CO₂ 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.

4.2. CO₂ impact on autoimmune antigen-antibody "Ag-Ab" reaction & Epitope-paratope

Our study investigated CO₂ chemical effect on autoimmune interaction. The CO₂ chemo-reflex is required for tonic drive underpinning electrostatic forces and paratope-epitope bonding. The chemical autoimmunity induced by chemical bonds is catalyzed by CO₂ rise; In hope of gaining a better understanding of how CO₂ 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 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 nanometers 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 pH 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 pH 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 et al, demonstrated a high correlation between corresponding structural properties of paratope and epitope [49]. Stank et al, 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 catalyzes 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 catalyzed initiating, maintaining, and providing an optimum temperature and pH, mediated by receptors, proteins and signaling 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 et al, 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].

4.3. CO_2 impact 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 CO₂ pressure reduces pH, which increases extracellular adenosine concentration [55]. These signaling events contribute to a range of physiological responses, some of which are sustained and involve CO₂-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 CO₂ is reflected on binding topologies of TCR-MHC and the observed differences between autoimmune complexes and anti-microbial complexes as demonstrated by Wucherpfennig et al [57]. It is the CO₂ 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 CO₂ on other immune cells.** Inflammation negatively regulates memory precursor effector cell development (MPEC). Notably, we are aware that elevated CO₂ generates anti-inflammatory effects via acidification. Elevated CO₂ 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 CO₂ in driving B cell autoimmunity. This role is concluded from Soboloff et al and Raza et al. By acting as sensors of temperature increases and acidosis, Ca²⁺ 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, CO₂ can promote B cell autoimmunity through its acid-thermic effects on Ca²⁺ Channel STIM1/Orai1- autophagy pathway.

The 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 et al, demonstrated that somatically mutated high-affinity autoantibodies are a hallmark of autoimmune diseases [62]. Jaiswal et al, 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 et al 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, demonstrated that certain proteins which modulate B cell polarity could modulate B cell responses to antigens and the MHC genes (the chief determinant for autoimmunity) could interact through their antigenic epitopes using the same Ig V genes [65]. Consequently, CO₂ 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 “CO₂” on cytosine deamination. Also, CO₂ 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 CO₂ on N-terminal α-amino groups and it is considered part of CO₂ sensing and signaling mechanism [66]. Furthermore, Casalino et al, found that CO₂ 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 CO₂ 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 CO₂ promotes emergence of autoimmune disorders in a pH-dependent manner.

In summary, multiple sites within the immune system can elicit adaptive responses to local CO₂ concentrations. The evidence points to strong contribution of acid/pH CO₂ sensing to modulate immune response. In this context, there is ample evidence that CO₂ exerts changes on catalytic antibodies towards benefit of autoimmunity.

4.4. 4.4. CO₂, T-cell activation, and function

Autoimmune induction by thermo-acidic effects unique to CO₂, 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. CO₂ modulates cellular cholesterol accumulation, and upregulation of lipid metabolism genes [68]. Robinson, et al, showed that cholesterol enriched in plasma membrane forms signaling 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 signaling following ligand engagement [70]. Unexpectedly, Gaud et al, found that T cells expressing TCRs containing inactivated CD3 ζ ITAMs (6F-CD3 ζ), exhibited reduced ability to discriminate between low- and high-affinity ligands, resulting in enhanced signaling and cytokine responses to low-affinity ligands. Also, TCRs were refractory to antagonism. Finally, T cells expressing 6F-CD3 ζ 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 et al, 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 Ca²⁺ may compete with negatively charged phospholipids, thus releasing CD3 ITAMs from plasma membrane [73].

In summary, CO₂ 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, CO₂ 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 signaling 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 Ca²⁺ responses to TCR stimulation for acquisition of T cell full functional competence to avoid autoimmunity. The outcome would blunt immunity. 5th, Yang et al [75], discussed that low pH inhibits IFN γ → upregulated Th17 cells which are involved in pathogenesis of diverse autoimmune diseases.

In conclusion, CO₂ plays a major role in orchestrating the immunologic response after a major insult of disturbed Ca²⁺ homeostasis.

Other studies have yielded contradictory findings to our conclusion. A meta-analysis stated that Methylene tetrahydrofolate 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 NH₃ and CO₂ from urea. Gradual build-up of CO₂ 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 CO₂-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 Ca²⁺ influx which prolongs IL-8-induced CO₂ 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 et al, 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.

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.
- Transient receptor potential channels = TRPCs
- 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 signaling.
- APCs = Antigen presenting cells.
- MHC = Major histocompatibility complex.
- TCRs = T cell receptors.
- ITAMs = Immune-receptor Tyrosine-based-Activation-Motifs

5. 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.

- CO₂ penetrates cell membranes to act as an electric drill with the result of forcing Ca² to get inside the cells against their concentration gradient “CO₂ drill like action”. This increased intracellular calcium influx augments the second messenger CO₂ signal that just has made by its immediate intracellular entry. The CO₂ 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 CO₂, 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 CO₂ thermal cytosine deamination and protonation, to dissociate phosphate groups which attack cytosine amino group. On the other hand, the CO₂ induction of mutated antibodies may be further catalyzed by the “MHC” susceptibility genes for autoimmune diseases.

Future prospectives

- Continued investigation of the role of CO₂ 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 CO₂ pathways along with prediction of possible mutations and their effects on immune response must be considered.
- The pivotal role of CO₂ 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 CO₂ 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 CO₂ 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 CO₂ rise and to compete with its intracellular diffusion by employing hyperbaric oxygen to neutralize CO₂ 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 Ca²⁺ channels and their precise targeted drug delivery, we must ensure the specificity of developed drugs for the Ca²⁺ 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 Ca²⁺ 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.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The authors declare no conflict of interest.

Statement of informed consent

Informed consents were obtained from participants included in the study.

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