Personalized-Inherent Variability in a Time-Dependent Immune Response: A Look into the Fifth Dimension in Biology

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Abstract
Introduction: Individualized response to the immune triggers influences the course of immune-mediated diseases and the response to immunotherapies. Both inter- and intra-subject variations occur in time-dependent dynamics of biological systems. The present study aimed to establish a model for inherent personalized-time-dependent variability in response to immune triggers. Methods: Male C57BL/6 mice were administered concanavalin A (ConA) and followed every 2 h for 10 h and at 24 h for serum alanine aminotransferase (ALT) levels. Results: A marked intragroup variability was noted for both the timing of the effect of ConA, the magnitude of the increase in ALT levels, and the time to peak. While in some mice, a peak level was achieved, whereas a continuous increase in liver damage was noted in others. Four mice died at different time points during the study irrespective of their liver damage, further supporting the individualized-based response to the trigger. Conclusions: This feasibility study established a model for determining the personalized-inherent variability in a time-dependent response to the immune triggers. These results highlight the importance of considering both the time and the wide range of individualized variability in immune responses while designing personalized-based immunotherapies.

Introduction

Biological systems have a dynamic course, which is affected by multiple parameters. These systems should not be viewed as being “all or none processes,” wherein an intracellular event or whole organ functions result from an “on-off button”-associated event [1]. Time-dependent dynamics have both intra- and inter-subject variabilities. Both time-dependent dynamic and threshold responses have been described for the immune system. Individualized response to immune triggers exerts an effect both on the course of immune-mediated diseases and on the response to immunotherapies. A continuous gradual process was described for lymphocyte function. CD8+ T-cell exhaustion is a process that involves a gradual decrease in their effector function, occurring during persistent antigen stimulation [2]. This process impairs the re-
sponse to infections and malignancy and is controlled by transcription factors such as the nuclear factor of activated T cells (NFAT), which promotes T-cell energy and exhaustion [2]. Immune remodeling is a dynamic process that refers to a course of deterioration and repair, which happens during acute and chronic immune-associated diseases [3]. Immune remodeling is part of a beneficial chronic dynamic adaptation required for promoting healthy survival.

The acute process poses a risk of exhaustion of the immune repertoire [3]. In the macaque AIDS model, a gradual decrease in SIV-specific CD8+ T-cell frequencies is associated with infection [4]. A gradual T-cell outgrowth occurs in chronic lymphocytic leukemia xenograft models, affecting tumor biology [5]. Tumor suppressor gene in transformed follicular lymphoma identified on 6q25 showed a gradual and persistent posttranscriptional regulator that attenuates local inflammation in the CNS by suppressing the infiltration of Th17 cells during the resolution phase of experimental autoimmune encephalitis, which contributes to recovery from T-cell-mediated autoimmune diseases [6]. Acute kidney injury after septic shock is driven through interleukin 17 (IL-17) released by Th17 cells. A gradual decrease of IL-17, but not of IL-10, IL-22, and interferon-gamma (IFNγ) from kidney homogenates, occurs under these conditions [7].

A similar gradual continuum process was described at the genetic level. A stochastic cell fate process in Bacillus subtilis sporulation mutants was described in which a gradual adjustment of genetic parameters allows genetic control of the penetrance of multiple fates [8]. In several biological systems, a continuing change to a threshold is described. A gradual accumulation of cyclin in a frog egg induces an abrupt activation of mitosis-initiating factors. Activation is delayed even after the accumulation of cyclin to a critical threshold concentration [9]. This abrupt activation follows a lag period independent of the concentration of cyclin.

The present feasibility study aimed to establish a model for inherent personalized-time-dependent variability in response to immune triggers. Concanavalin A (ConA), which induces immune-mediated hepatitis, was used as an immune trigger in this study [10–13].

**Methods**

**Animals**

Male C57BL/6 mice, aged 11-12 weeks, were obtained from Envigo Laboratories (Jerusalem, Israel) and maintained in the Animal Core of the Hadassah-Hebrew University Medical School. Mice were administered standard laboratory chow and water ad libitum and kept in a 12-h light and dark cycle. Animal experiments were carried out according to the guidelines of the Hebrew University-Hadassah Institutional Committee for Care and Use of Laboratory Animals. The joint Ethics Committee (IACUC) of the Hebrew University and Hadassah Medical Center approved the study protocol for animal welfare. The Hebrew University is an AAALAC International accredited institution.

**Induction of ConA Immune-Mediated Hepatitis and Follow-Up of Liver Enzymes**

ConA (MP Biomedicals, Irvine, CA, USA) was dissolved in 50-mM Tris pH 7, 150-mM NaCl, 4-mM CaCl₂ and injected into the tail vein at a dose of 500 μg/mouse (15 mg/kg body wt). To study the effect of a trigger on the immune system, eight mice were injected with ConA and followed every 2 h for 10 h and at 24 h for measuring the serum alanine aminotransferase (ALT) levels using an automatic analyzer (Beckman Coulter). Mice were administered standard laboratory chow and water ad libitum and kept in a 12-h light and dark cycle following the ConA injection.

**Statistical Analysis**

Statistical analysis was performed using the Student’s t test. A p value <0.05 was considered significant.

**Results**

The effect of the immune system on a trigger was determined by analyzing serum ALT levels after the injection of ConA. Figure 1 and Table 1 summarize the ALT levels in individual mice when measured every 2 h for 10 h and at 24 h following the injection.
Marked intragroup variability was noted both for the timing of the effect and the magnitude of the liver damage as measured by the serum ALT levels. The ALT levels were 108–234 u/L at 2 h and 4,440–14,160 u/L with maximal levels at 8–10 h following injection. Marked variability was also observed for the time to the peak damage. While in some mice, a peak level was achieved, whereas in others, a continuous increase in liver damage was noted. A drop-in hour 4 was noted for some of the animals.

Four mice have died at different time points during the study, which is a well-described cytokine-storm-associated phenomenon of the mouse model [14]. This was irrespective of the liver damage and suggested a different individualized time-dependent response to the trigger.

Discussion

In this proof-of-concept feasibility study, we establish a model for an individualized response to an immune trigger, manifested both in the time and magnitude of the immune response. ConA was used as the immune trigger in an isolated system which enabled the follow-up of an individualized time-dependent gradual development of immune-mediated liver damage since all mice in the current study were genetically identical and handled under similar conditions; however, there were marked differences in their immune responses. The remarkable differences in response time might be due to the differences in the magnitude of the effects on individual animals.

A better understanding of biological complexity requires investigating cellular behavior by culturing cells in multiple dimensions [15]. Culturing cells in three-dimensional (3-D) systems rather than in two-dimensional flat-dishes is much closer to exhibiting their physiological behavior in vivo [16]. Time is described as the 4th dimension, which is not always being taken into consideration in cellular and molecular biology experiments [15]. There are several dynamic phenomena in biological systems that are time-dependent. Including time as the fourth dimension into a biological system is critical for a better understanding of its function, kinetics, and signaling [15].

Considering time in biological processes is equally essential for monitoring disease progression and drug discovery [1]. The continuity of a biological process and the realization that they are on a scale of an ongoing course are of great importance for all data interpretation.

Variability in biological clocks among individuals may underlie some of the findings in the present study. The data suggest that these clocks work differently among seemingly identical individuals. An individualized “Chrono-immunology” is suggested to play a role in the differences noted among the mice studied. Chronobiology studies circadian timing at both the organismal and cellular levels [17, 18]. The circadian clock is an endogenous timing system that controls many physiological processes [17, 19]. Altered circadian timing systems are linked to molecular dysfunctions and may result in severe pathologies, including cancer [17, 19].

Time is of relevance for immunology and metabolomics analyses [20]. The importance of time in immune metabolism is reflected by the stepwise adaptation of metabolism to sustain the bioenergetic demand of an immune response [21]. The adapted inflammation metabolism is specific concerning the effector function and is a well-arranged dynamic event, which involves a time-dependent interplay of signaling and metabolic pathways [21]. Time plays a role in infiltrating immune cells into tissue affecting their function [21].

T-cell recognition involves a formation of an immunological synapse between a T cell and a target cell, result-

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Table 1. ALT serum levels (IU) per hour following ConA injection

| Hours post-ConA injection | Animal No. | 0   | 2   | 4   | 6   | 8   | 10  | 24  |
|--------------------------|------------|-----|-----|-----|-----|-----|-----|-----|
|                          | 1          | 5   | 150 | 5   | 5   | 1,776 | 4,440 | 2,080 |
|                          | 2          | 5   | 139.6 | 101.4 | 151.4 | 3,320 | 6,340 | 5,980 |
|                          | 3          | 5   | 234 | 5   | 3,320 | 14,160 | 13,080 | D   |
|                          | 4          | 5   | 195.4 | 5   | 240 | D   | D   | D   |
|                          | 5          | 5   | 108.6 | 5   | 500 | 3,500 | 4,760 | 8,180 |
|                          | 6          | 27.9 | 152.4 | 5   | 150.4 | 3,760 | 5,180 | 4,660 |
|                          | 7          | 5   | 155.4 | 116.4 | D   | D   | D   | D   |
|                          | 8          | 5   | 198 | 181.4 | 1,706 | 10,280 | D   | D   |
| Av                       | 7.9        | 166.7 | 112.4 | 881 | 6,132.7 | 6,760 | 5,225 |

D, died and not tested.
ing in cytotoxicity of a target cell by CD8 cells or cytokine released from proliferating helper CD4 cells. Determining the synapses of antigen-specific CD4 cells, which recognize B cells and dendritic cells at different time points, exhibit several time-dependent stages in synapse formation, proceeding over several hours [22]. Stage-dependent dynamic changes also occur at the level of the intracellular apparatus, including centrioles and the Golgi complexes, which are located beneath the synapse, supporting the importance of time in these events [22].

Mathematical modeling and quantitative experimentation are developed to introduce time as a component into isolated systems [23]. This was used, for example, in studying the control systems for molecular signals which act on cells in embryos to generate patterns that govern the timing of developmental events [23]. A structural basis for protein conformational dynamics at high resolution was described [24]. At a cellular level, studies of the path of carbons during photosynthesis employed the kinetics of radioactivity in these systems [25]. Similarly, the kinetics of the path of carbon in glucose metabolism is fundamental for the system’s function [26, 27]. Adding the time dimension enabled identifying glucose transport as a rate-limiting step, rather than its phosphorylation, and determining the differences in aerobic glycolysis between normal and virus-transformed cells as a consequence of the difference in the rates of glucose uptake [26, 27].

At the level of whole organs, rhythmic motor behaviors, including walking and micturition, are episodic and exhibit a gradual run-down in frequency before spontaneously terminating. A gradually changing balance between ATP and adenosine underlies this run-down of the motor patterns [28]. Time plays a role in nonevolutionary studies of cross-species comparisons and identifying the target gene or protein involved in functional characterization [29]. The drug-target residence time model is essential for conformational dynamics of target macromolecules that affect drug bindings and dissociations [30].

Looking into the “3rd and the 4th dimensions” does not finish the quest to comprehend biological systems [15]. Our findings suggest that in addition to time, adding an individualized variability, viewed as “the fifth dimension,” is required for improved analysis of isolated systems [31–34]. Considering an individualized response, which involves both the time and the magnitude of the response, rather than looking into means of the group, maybe needed for better design of precision medicine [35].

No two organisms are identical, and multiple variables are associated with differences in the responses. These may also underlie a difference in response in terms of timing. Personalized variability or randomness in isolated biological systems may underlie the present study results. An inherent variability was described in various biological systems, including heart, breathing, and gait [36]. Loss or change in the expected variability in some organs, such as the heart, is associated with poor prognosis [37–39]. Biological randomness can be viewed as an essential component of the heterogeneous determination and intrinsic unpredictability proper to organisms, the interaction between many levels of the organization, and a key component of its structural stability [40]. While increasing “order,” the increasing organization induces growing disorder by energy dispersal effects and by increasing variability and differentiation [40]. Applying some of these concepts to therapeutic measures was suggested [41–56].

At this time, the response to therapy is assessed in large populations, and the present data suggest looking into more individualized methods for assessing response [31, 32, 53]. Second-generation artificial intelligence systems are being developed to provide potential solutions to the problem of individualized responses to therapies [31, 32].

In summary, the data of the present feasibility study establish a model for determining the personalized-inherent variability in response to immune triggers. The described variability involves both times and magnitudes of the immune response. These findings support high variability in time-dependent immune-mediated manifestations between subjects, which need to be considered while designing personalized-based immunotherapies.

**Statement of Ethics**

Animal experiments were carried out according to the guidelines of the Hebrew University-Hadassah Institutional Committee for Care and Use of Laboratory Animals. The joint Ethics Committee (IACUC) of the Hebrew University and Hadassah Medical Center approved the study protocol for animal welfare. The Hebrew University is an AAALAC International accredited institution (approval date 12/2018).

**Conflict of Interest Statement**

The authors have no conflicts regarding the present study. Yaron Ilan is the founder of Oberon Sciences.

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