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Bioactive compounds in green tea may improve transplant tolerance: A computational systems biology analysis

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SUMMARY

Background: Green tea (*Camellia sinensis*) has bioactive compounds that have been shown to possess nutritive effects on various biomolecular processes such as immunomodulation. This research explores the immunomodulatory effects of green tea in reducing transplant rejection.
Method: The study employs computational systems biology: 1) to identify biomolecular mechanisms of immunomodulation in transplant rejection; 2) to identify the bioactive compounds of green tea and their specific effects on mechanisms of immunomodulation in transplant rejection; and, 3) to predict the quantitative effects of those bioactive compounds on immunomodulation in transplant rejection.
Results: Three bioactive compounds of green tea – epicatechin (EC), gallic acid (GA), and epigallocatechin gallate (EGCG), were identified for their potential effects on immunomodulation of transplant rejection. Of the three, EGCG was the only one determined to enhance anti-inflammatory activity by: 1) upregulating synthesis of HO-1 that is known to promote Treg and Th2 phenotypes associated with enabling transplant tolerance; and, 2) downregulating pro-inflammatory cytokines IL-2, IL-17, IFN-γ, TNF-α, NO, IL-6, and IL-1β that are known to promote Th1 and Th17 phenotypes associated with transplant rejection.
Conclusions: To the best of our knowledge, this study provides the first molecular mechanistic understanding the clinical nutritive value of green tea, specifically the bioactive compound EGCG, in enabling transplant tolerance.

1. Background

Green tea is consumed globally and contains several bioactive compounds that are potent anti-inflammatories [1]. Persistent inflammation adversely affects host immune response following solid organ transplantation, resulting in multiple organ failures and, in some cases, death [2]. The anti-inflammatory effects of bioactive compounds in green tea may beneficially affect immunomodulation and reduce transplant rejection.

1.1. Green tea

Green tea is a boiling water extract prepared with the leaves of *Camellia sinensis* L., an evergreen shrub of the Theaceae family. Green tea has been traditionally consumed in China, Japan, India, and a few countries in North Africa and the Middle East [3]. It is the second most popular beverage worldwide that is also a major source of dietary flavonoids. It is also known for its medicinal and health benefits in the eastern traditional systems of medicine since ancient times [4] because of its anti-proliferative, anti-mutagenic, antioxidant, antibacterial, anti-viral, immunoregulatory, and chemopreventive properties [5]. Additionally, several of the bioactive compounds in green tea have been shown to possess strong anti-inflammatory and immunomodulatory properties [6,7]. Immunomodulation is a key driver of success in solid organ transplantation.

1.2. Transplant rejection

Tissue and organ transplantation have become a standard practice to treat organ failure. In most transplantation cases, there is a substantial risk of transplant rejection as the donors and recipients may not be perfectly matched [8]. Adverse immune

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response in the recipient can lead to transplant rejection and prove fatal, especially in kidney, heart, or lung transplantation [9]. This transplant rejection is primarily coordinated by the adaptive immune system through the T cells of the recipient [10].

Current clinical practice to overcome transplant rejection includes immunosuppressive drugs such as mycophenolic acid, calcineurin inhibitor, azathioprine, and corticosteroids. Combinations of these drugs are administered at a higher dosages in the early post-transplantation period, and at a lower dosage over a longer period of time in the post-transplantation period [11]. These drugs, however, have severe side effects and can cause cardiovascular disease, cancer, and nephrotoxicity. Recent research efforts have focused on finding alternative immunosuppressants with less severe side effects; molecules that suppress donor specific antibodies; and, biologics that only bind to the immune cells thereby minimizing toxicity [9]. Therapeutic interventions promoting improved tolerance of transplanted organs such as costimulatory blockade agents, depletion of T cells, and Treg therapy, have been shown to effectively reduce the need for long-term immunosuppressants [12], and are gaining wide acceptance in clinical practice.

1.3. Immunomodulation in transplant rejection

In the host, T cells exert their reactive immune response through several molecular mechanisms including direct recognition of alloantigen from the donor antigen presenting cell (APCs); recognition of processed allopeptide by the recipient's APCs; and, recognition of intact alloantigen presented by recipient APCs [10]. Tregs, on the other hand, inhibit immune response from effector T cells by competing with T cells for IL-2; mediation of cAMP signaling in T cells; and, mediation of adenosine receptor signaling in T cells [13–15]. Balance between reactive effector T cells and cytoprotective Treg cells is critical in modulating transplant rejection [16]. This balance is strongly influenced by the inflammatory state in the host following transplant [9]. A highly pro-inflammatory environment in the newly transplanted organ can direct T cell differentiation to the reactive Th1 and Th17 cells; whereas, in absence of pro-inflammatory cytokines, the naïve T cells may differentiate into cytoprotective Tregs [17]. A strategy for improving transplant tolerance, therefore, is to manipulate the inflammatory environment in the transplanted organ within the host by promoting Tregs differentiation.

1.4. Green tea and immunomodulation in transplant rejection

Green tea, especially its bioactive compound EGCG, has strong anti-inflammatory properties [6]. EGCG has been shown to have immunomodulatory effects on CD4+ T cells and NK cells [7]. EGCG's effect on immune pathologies [18,19], inflammatory disease [1], and cancer [20] is well documented. In an earlier work, EGCG in combination with quercetin, has been shown to ameliorate transplant-related organ damage [21]. Given inflammation plays a significant role in post-transplantation organ damage, and given the increasing evidence of green tea's bioactive compounds in reducing inflammation, the immunomodulatory effects of administering green tea following organ transplantation need further investigation.

1.5. Systems biology approach

The molecular mechanisms of action of greet tea bioactive compounds on transplant rejection are not well understood through conventional *in vitro* and *in vivo* methods of investigations. Modern systems biology, employing bioinformatics and computational methodologies, provides an opportunity to identify the molecular mechanisms of action as well as to create quantitative mathematical models to determine the effect of bioactive compounds on immunomodulation of transplant rejection. In this study, we employ a computational systems biology methods [22] to: 1) to identify biomolecular mechanisms of immunomodulation in transplant rejection; 2) to identify the bioactive compounds of green tea and their specific effects on mechanisms of immunomodulation in transplant rejection; and, 3) to predict the quantitative effects of those bioactive compounds on immunomodulation in transplant rejection. Previous work has demonstrated the viability of using such computational systems biology approach to model complex biomolecular phenomena [23–25].

2. Materials and methods

This section describes the methodology used to identify the molecular mechanisms of immunomodulation in transplant rejection and to determine the effects of bioactive compounds of green tea on such mechanisms.

2.1. Process

There are five (5) steps in this process, as illustrated in Fig. 1a, and itemized below:

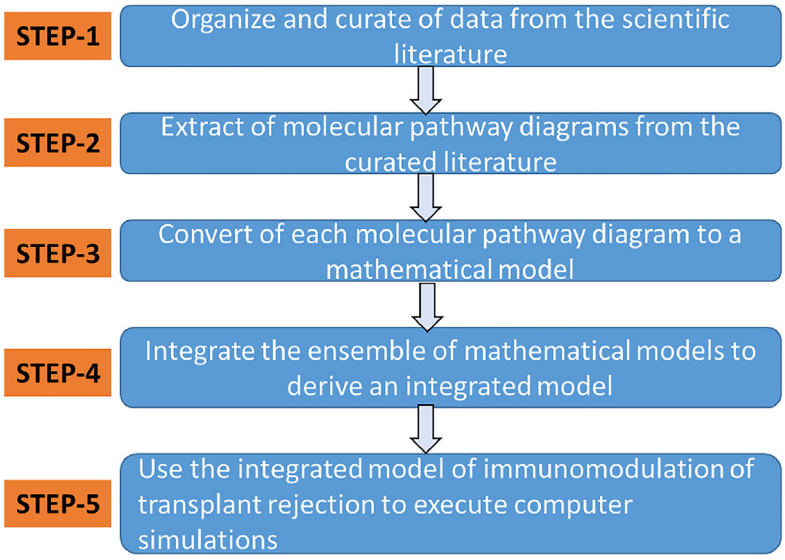
1) Organize and curate data from the scientific literature
2) Extract molecular pathway diagrams from the curated literature
3) Convert each molecular pathway diagram to a mathematical model
4) Integrate the ensemble of mathematical models to derive an integrated model of immunomodulation of transplant rejection
5) Use the integrated model of immunomodulation of transplant rejection to execute computer simulations

2.2. Organize and curate data from the scientific literature

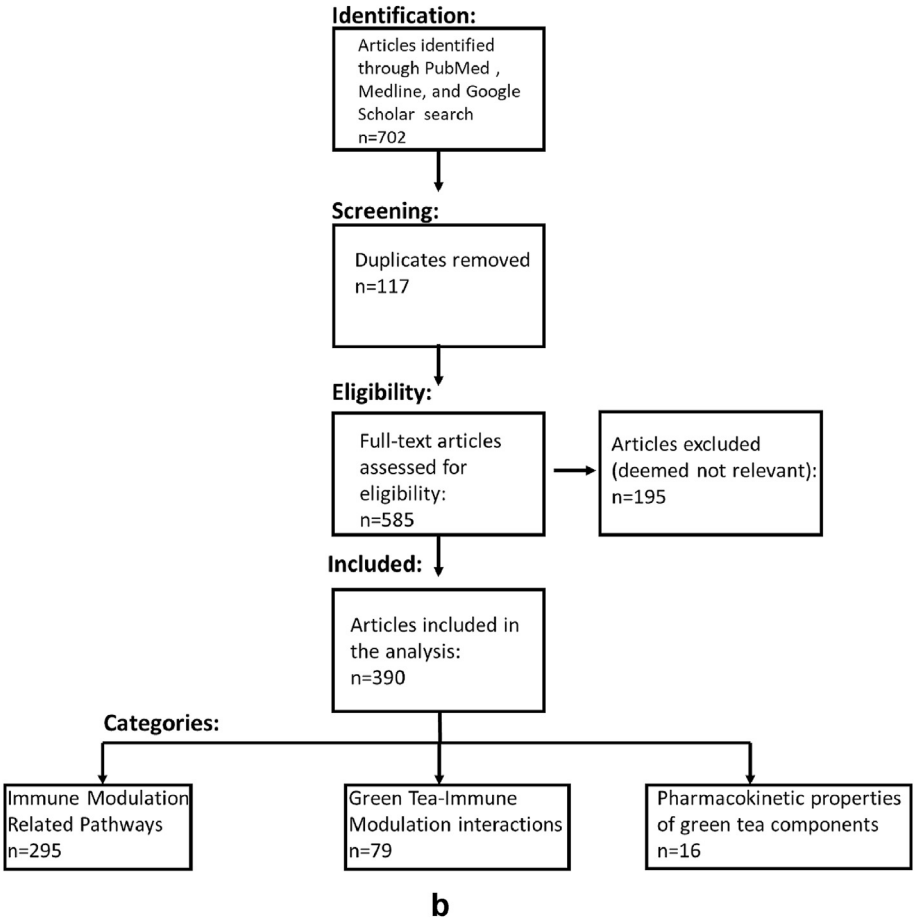
The scientific literature is searched to identify journal papers that contain research on immunomodulation of transplant rejection, molecular pathways of immunomodulation of transplant rejection, bioactive compounds in green tea, and the effect of bioactive compounds in green tea on immunomodulation of transplant rejection molecular pathways. Four (4) steps are necessary to organize and curate the journal papers, as itemized below:

1. Create a list of Medical Subject Headings (MeSH) keywords to optimize recall and precision of peer-reviewed articles
2. Search and retrieve the relevant peer-reviewed articles published between January 1985 to May 2018 from PubMed, Medline, and Google Scholar. These set of articles are stored as an “Initial Set” repository
3. Screen the titles and abstracts of articles in the Initial Set repository to identify most relevant articles based on our inclusion criteria. These set of articles are stored as the “Final Set” repository
4. Perform full-length review of peer-reviewed articles from the Final Set repository

Abstracts and unpublished literature were not sought as they have not been peer reviewed adequately to authenticate their results. The literature review inclusion criteria and categorization process are represented in the form of PRISMA diagram in Fig. 1b.



a



b

Fig. 1. **a**. Methodology used to identify the molecular mechanisms of immunomodulation in transplant rejection and to determine the effects of bioactive compounds of green tea on such mechanisms; **b**: PRISMA flow diagram.

The journal articles included in the Final Set were subjected to full-length review and were classified into three groups:

Group 1) Articles on immunomodulation of transplant rejection pathways;
Group 2) Articles on green tea bioactive compounds interacting with immunomodulation of transplant rejection pathways; and,
Group 3) Articles on pharmacokinetic properties of green tea bioactive compounds.

2.3. Extract molecular pathway diagrams from the curated literature

Journal articles in Group 1 are reviewed to gather data relevant to molecular pathways of immunomodulation of transplant rejection. The steps to extract and represent molecular pathways diagrammatically are itemized below:

1. Identify and extract:
   a. Chemical species involved in immunomodulation of transplant rejection
   b. Types of cells involved in immunomodulation of transplant rejection
   c. Cellular components (e.g. cytosol, mitochondria, nucleus, etc.) where the chemical species are present in each cell type
2. Identify and diagrammatically represent biochemical interactions in immunomodulation of transplant rejection
3. Interconnect biochemical reactions to create molecular pathway diagram in each cell type involved in immunomodulation of transplant rejection

The list of chemical species and the biochemical reactions are provided in Tables C1–C7 of Appendix C in the Supplementary Information.

Journal articles in Group 2 are reviewed to gather data relevant to bioactive compounds of green tea. Following information is extracted:

1. Bioactive compounds of green tea
2. Concentration levels of bioactive compounds in green tea

Bioactive compounds and their concentration levels are provided in Table A2 of Appendix A.

Journal articles in Group 3 are reviewed to extract following information:

1. Pharmacokinetics of bioactive compounds of green tea
2. Reaction rate constants of biochemical reactions between bioactive compounds of green tea and their molecular targets in the immunomodulation of transplant rejection molecular pathways

This information is provided in Tables C1–C7 of Appendix C in the Supplementary Information.

2.4. Convert each molecular pathway diagram to a mathematical model

The steps to convert molecular pathway diagrams to mathematical models are itemized below:

1. Convert biochemical reactions involved in each of the molecular pathway into ordinary differential equations (mathematical expressions that describe the rate of change)
2. Represent each molecular pathway of immunomodulation of transplant rejection as a system of ordinary differential equations
3. Encode the system of differential equations in a computer software source code format known as Systems Biology Markup Language (SBML) [62] to construct a mathematical model for a particular molecular pathway of immunomodulation of transplant rejection
4. Store each model as a separate SBML file

2.5. Integrate the ensemble of mathematical models to derive an integrated model of immunomodulation of transplant rejection

In order to create an integrative quantitative model of immunomodulation of transplant rejection, it is necessary to mathematically couple the solutions across the ensemble of individual molecular pathway models. Such mathematical coupling is performed using the CytoSolve [37,38] computational engine, which is described in detail in Ayyadurai and Dewey, 2011 [61]. The computational architecture of CytoSolve enables the integration of plurality of molecular pathway models [37,61].

The CytoSolve architecture provides the following software layers to enable the coupling of multiple molecular pathway models to produce the integrative solution:

2.5.1. Presentation Layer: The Presentation layer provides an interface to the user where the individual molecular pathway models are uploaded for their dynamic integration. Another important feature of the Presentation layer is that it resolves any nomenclature conflicts in the molecular species and annotates biochemical reaction duplicates across the individual molecular pathway models and prepares them for integration by the Controller.

2.5.2 Controller Layer: In the Controller layer, computations are performed using the integrated model to arrive at the integrated solution. The Controller layer has three components which participate in deriving the integrated solution: the Monitor, the Communication Manager, and the Mass Balance. The Monitor tracks the completion of calculations by each model at every time step. The Communication Manager controls instructs initiation as well as pausing of calculations by each model for every time step. The Mass Balance ensures mass conservation of all molecular species as it integrates the calculations from the ensemble of models for every time step.

2.5.3. Communication Layer: The Communication layer facilitates messaging between the Controller and Models. For example, the Controller may send the input values of molecular species at time $t = n$ (S_n) and an instruction to a model to run a calculation over a time step. After performing the calculation for that time step, the model may pass the solution for that molecular species at time $t = n+1$ (S_{n+1}) back to the Controller. Such messages can be performed in parallel between the multitude of individual models and the Controller.

2.5.4. Database Layer: The Database layer has two components: Solutions and Ontology. Solutions stores the memory that tracks molecular species concentrations across all models for every time step. Ontology manages the molecular species nomenclature and biochemical reaction duplicates across all the models and ensures consistency while the Controller is calculating the integrated solution.

2.5.5. Model Layer: The Models layer houses the individual molecular pathway models. The CytoSolve architecture is designed in such a way that the individual models may reside on different servers, vary in complexity, and may have

different computational source code formats (e.g. SBML, CellML, MATLAB, etc.).

The steps to integrate the ensemble of mathematical models of immunomodulation of transplant rejection are listed below:

1. Upload individual SBML files, constructed in Section 2.4, to CytoSolve engine
2. Update the initial conditions for the molecular species in all the mathematical models in the graphical user interface
3. Update simulation period was specified in the graphical user interface
4. Review and confirm molecular species and reaction duplicates across all the immunomodulation of transplant rejection models in the graphical user interface
5. Commence integration of individual models of immunomodulation of transplant rejection

2.6. Use the integrated model of immunomodulation in transplant rejection to execute computer simulations

The effect of individual bioactive compounds and combination of the bioactive compounds in green tea was assessed by estimating the cellular concentration levels of biomarkers of immunomodulation of transplant rejection mechanisms that included TNF-α, IFNγ, nitric oxide (NO), IL-17, IL-2, XMP, IL-1β, Foxp3, and HO-1 in presence and absence of the green tea supplementation. The following computer simulations were performed:

1. Effect of EGCG on IL-12 mediated synthesis of pro-inflammatory biomarkers TNF-α and IFNγ in CD4+ T cells. These biomarkers promote Th1 and Th17 phenotypes and subsequent transplant rejection.
2. Effect of EGCG on IL-6 mediated synthesis of pro-inflammatory biomarker IL-17 in CD4+ T cells. IL-17 promotes Th17 phenotype and subsequent transplant rejection.
3. Effect of EGCG on TNF-α/STAT5 mediated synthesis of anti-inflammatory biomarker Foxp3 in naive T cells. Foxp3 promotes Treg phenotype and the subsequent transplant tolerance.
4. Effect of EGCG on TNF-α/NFkB mediated synthesis of pro-inflammatory biomarkers NO and IL-6 in naive T cells. These biomarkers promote Th17 phenotype and subsequent transplant rejection.
5. Effect of EGCG on TCR mediated synthesis of pro-inflammatory biomarker IL-2 in naive T cells. IL-2 promotes Th1 and Th17 phenotypes and subsequent transplant rejection.
6. Effect of EGCG on IMPDH mediated synthesis of pro-proliferation biomarker XMP in T cells. This biomarker promotes T cell proliferation and subsequently supports transplant rejection.
7. Effect of EGCG on HMGB1 mediated synthesis of pro-inflammatory biomarkers TNF-α, IL-1β, and IFNγ in dendritic cells. These biomarkers promote Th17 phenotype and subsequent transplant rejection.
8. Effect of EGCG on HSP60 mediated synthesis of pro-inflammatory biomarkers TNF-α, IL-1β, and IFNγ in dendritic cells. These biomarkers promote Th17 phenotype and subsequent transplant rejection.
9. Effect of EGCG on Nrf mediated synthesis of HO-1 in dendritic cells. HO-1 promotes Treg and Th2 phenotypes and subsequent transplant tolerance.

2.7. Computational model inputs

The following steps are executed to setup the inputs for the computational model of immunomodulation in transplant rejection:

1. Input biochemical reactions for interaction between bioactive compounds of green tea and immunomodulation of transplant rejection molecular pathways
2. Input the kinetic rate constants for each of the biochemical reaction
3. Input the initial concentrations for each of the molecular species in the biochemical reactions
4. Input the time period for the simulation of integrative models, and dose levels of bioactive compounds in green tea
5. Execute the updated integrative model of immunomodulation of transplant rejection

The list of biochemical reactions, the rate equations, and the kinetic rate constants of the biochemical reactions, and initial concentrations for the molecular species involved in the biochemical reactions are listed in Appendix C.

The concentration levels of bioactive compounds in green tea and Cmax (maximum plasma concentrations) of green tea bioactive compounds were obtained from the *in vivo* pharmacokinetic study performed by Lee et al., 2002 [23]. These concentration levels are used in the simulations to study the effect of green tea bioactive compounds on the nine inflammatory processes governing the transplant rejection. The parameter values are provided in Table 1. The simulations were performed for a duration of 200,000 seconds (s). This duration was selected since the concentration levels of all nine (9) biomarkers of interest achieved a steady state at 100,000 s to 180,000 s.

2.8. Computational model outputs

The following are the outputs from the computational model:

1. Time-dependent concentration of TNF-α
2. Time-dependent concentrations of IFNγ
3. Time-dependent concentrations of NO
4. Time-dependent concentrations of IL-17
5. Time-dependent concentrations of IL-2
6. Time-dependent concentrations of XMP
7. Time-dependent concentrations of IL-1β
8. Time-dependent concentrations of Foxp3
9. Time-dependent concentrations of HO-1

2.9. Analysis of computational model output

The following steps are performed to analyze the output from the computational model:

Table 1
Cmax values of EGCG, epicatechin and gallic acid.

Green tea dose (mg/kg)	EGCG Cmax (nM)	Epicatechin Cmax (nM)	GA Cmax (nM)
20	170	429	1.5

1. Export the raw data to Microsoft Excel
2. Extract the steady state levels of TNF-α, IFNγ, nitric oxide (NO), IL-17, IL-2, XMP, IL-1β, Foxp3, and HO-1
3. Plot steady state levels of TNF-α, IFNγ, nitric oxide (NO), IL-17, IL-2, XMP, IL-1β, Foxp3, and HO-1 in presence and absence of individual bioactive compounds in green tea

3. Results

This study provides three (3) results: 1) the molecular mechanisms of immunomodulation in transplant rejection; 2) the bioactive compounds of green tea and their specific effects on the molecular mechanisms of immunomodulation in transplant rejection; and, 3) the computational analysis of bioactive compounds of green tea on mechanisms of immunomodulation in transplant rejection.

3.1. Molecular mechanisms of immunomodulation in transplant rejection

A systematic literature review was conducted to identify the molecular mechanisms involved in immunomodulation of transplant rejection.

3.1.1. Systematic literature review

A systematic literature review resulted in the identification of an initial set of 702 articles. The intermediate and final results of the systematic review are summarized in Fig. 1b. Further analysis of the title and abstract yielded 390 relevant articles that were comprehensively reviewed by the authors. Of these 390 relevant articles, 295 informed about the nine molecular pathways related to immunomodulation of transplant rejection; 79 informed about the biochemical interactions between green tea bioactive compounds and the nine molecular pathways related immunomodulation of transplant rejection; and, 16 informed about the pharmacokinetic and pharmacodynamics properties of green tea bioactive compounds.

3.1.2. Molecular mechanisms identified in immunomodulation in transplant rejection

Nine molecular mechanisms involved in immunomodulation during transplant rejection, were identified. Seven pro-inflammatory molecular pathways that lead to transplant rejection were identified. Two anti-inflammatory molecular pathways that lead to transplant tolerance were identified.

Pro-inflammatory Pathways

I. **IL-12 mediated Th1 differentiation**: Expression of IL-12, an immunomodulatory cytokine, is elevated during the process of allograft rejection [26]. IL-12 promotes Th1 differentiation of CD4+ T cells. IL-12 triggers activation of Jak2, which in turn phosphorylates STAT4. STAT4 is a transcription factor responsible for expression cytokines such as IL-2, IFN-γ and TNF-α that promote the differentiation of Th1 cells. The sustained signaling of IL-12 mechanism creates an inflammatory environment that results in graft rejection after transplantation [26,27].
II. **IL-6 mediated Th17 differentiation**: IL-6 plays a key role in promotion of acute graft rejection. In naïve CD4+ T-cells, IL-6 initiates signaling cascade via JAK/STAT3/RORγt leads to expression of pro-inflammatory cytokines such as IL-17, IL-21, and IL-22, which promote the Th17 phenotype. Additionally, in concert with TGF- β, IL-6 was shown to promote the Th17 phenotype. IL-6 also has been implicated in promoting Th1 response, leading to transplant ejection [26–28].
III. **TNF-α signaling pathways**: TNF-α has is reported to initiate rejection response, especially during cardiac transplantation. Activated T cells initiate macrophage induced TNF-α release [29]. The signaling pathway by TNF-α via TNFR1 receptor initiates pro-inflammatory pathways that lead to increased expression of pro-inflammatory cytokine IL-17. IL-17 drives the promotion of Th17 phenotype and subsequent transplant rejection [30,31]. TNF-α binding to its receptor TNFR2 triggers proliferation of Treg cells via expression of TGF-β and IL-10, and consequently, graft tolerance [32].
IV. **TCR signaling pathway**: In graft rejection, antigen specificity for T cells is provided by TCR that leads to a strong immune responses to transplanted graft and rapid graft rejection [33]. TCR signaling induces the synthesis of IL-2, a potent pro-proliferative cytokine, via PLCγ1/PIP2/DAG/MKK/JNK-AP-1 signaling transduction pathway leading to an adaptive immune response against the graft [34,35].
V. **IMPDH metabolism**: IMPDH mediates de-novo purine synthesis which is crucial for proliferation of T cells [36]. Expression of the IMPDH-I mRNA is upregulated during the first 3 months following renal transplantation and that this induction is dramatically strengthened during acute rejection episodes [37,38]. Inhibiting IMPDH activity can thus reduce the proliferation of T-cells and can promote the setting of organ transplantation [39].
VI. **HSP60 induced TLR-4 signaling pathway**: During transplant, ligands such as extracellular matrix components and HSP60, bind to the TLR4 receptors on dendritic cells. TLR4 has been shown to play a critical role in renal graft rejection caused by ischemia-reperfusion injury via upregulation of pro-inflammatory cytokines such as IL-6, MCP-1, IFN-γ, IL-1β, TNF-α [40–42].
VII. **HMGB1 induced TLR4 signaling pathway**: During transplant, endogenous ligands like HMGB1 bind to the TLR4 receptors on dendritic cells. TLR4 has been shown to play a critical role in renal graft rejection caused by ischemia-reperfusion injury via upregulation of pro-inflammatory cytokines such as IL-6, MCP-1, IFN-γ, IL-1β, TNF-α [40–42].

Anti-Inflammatory Pathways

VIII. **TGF-β mediated Treg differentiation**: Treg cell activity plays an essential role in the success of transplantations. TGF- β promotes expression of regulatory cytokines TGF-β and IL-10 via SMAD3/4-Foxp3. TGF-β and IL-10 overexpression promotes Treg cell activity and favors graft survival [26,27,43].
IX. **Nrf2 Signaling pathway**: Anti-oxidative properties of Nrf2 mediated gene expressions may help in graft survival during transplantation. Nrf2 binds to antioxidant response element and initiates the expression of a group of detoxifying and antioxidant genes, such as Hemeoxygenase-1 (HO-1), glutathione S- transferase (GST) and NAD(P)H: quinone oxidoreductase-1 (NQO1) [30,31]. Upregulation of Nrf2 antioxidant pathway in dendritic cells also leads to the promotion of anti-inflammatory Tregs phenotype [44].

A *molecular systems architecture* – an integration of the nine aforementioned immunomodulatory mechanisms – is created to provide a systems-level understanding of immunomodulation in transplant rejection. This molecular systems architecture is illustrated in Fig. 2. Anti-inflammatory mechanisms are implicated in transplant tolerance by Treg and Th2 promotion, and by Th1 and Th17 suppression. Pro-inflammatory mechanisms are implicated in transplant rejection is by Treg and Th2 suppression, and by Th1 and Th17 promotion.

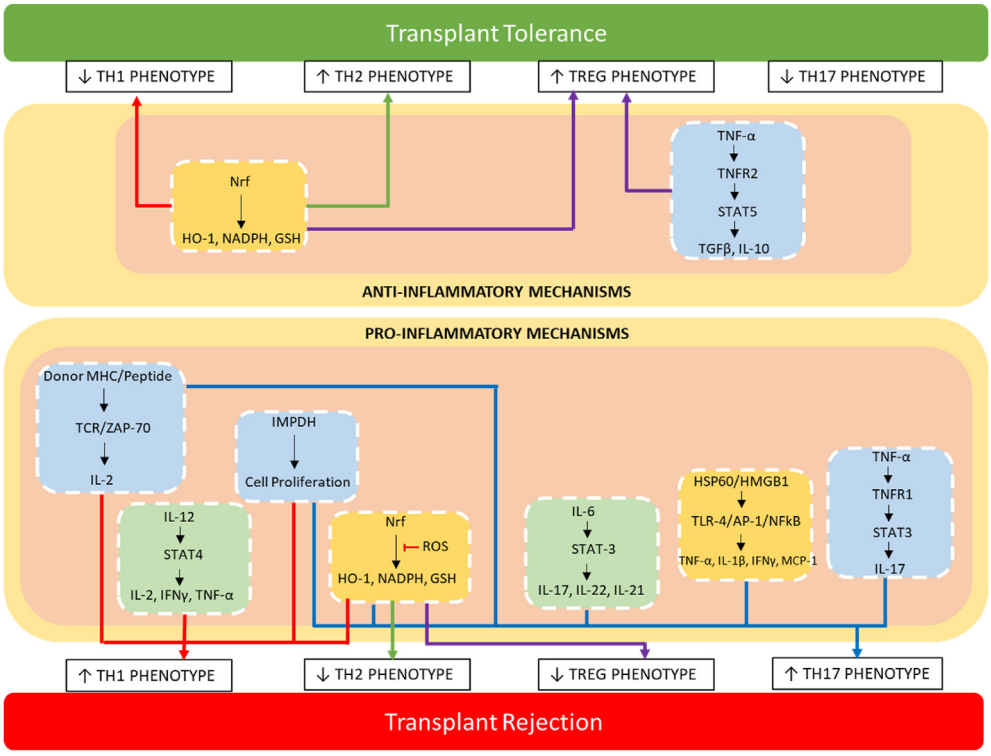


Fig. 2. Molecular Systems Architecture of Immune Modulation. Nine mechanisms, partitioned as anti-inflammatory and pro-inflammatory, were identified within three cell types: naive T cells (blue), CD4+ T cells (green), dendritic cells (yellow). The mechanisms serve to promote or suppress: Treg phenotype (purple arrows), Th1 phenotype (red arrows), Th2 phenotype (green arrows), and/or Th17 phenotype (blue arrows). Promotion of Treg and Th2 along with suppression of Th2 and Th17 supports transplant tolerance. Promotion of Th1 and Th17 along with suppression of Treg and Th2 enhances transplant rejection.

3.2. Green tea bioactive compounds and their interactions with immunomodulatory pathways

A systematic bioinformatics review identified the bioactive molecules of green tea, and the specific effects of those molecules on the mechanisms of immunomodulation in organ transplantation. These results are discussed in detail below.

3.2.1. Green tea bioactive compounds

Green tea is an infusion or a boiling water extract prepared with the leaves of *C. sinensis* L. and water, and a beverage of choice in many countries around the world. Green tea has been traditionally consumed in China, Japan, India, and a few countries in North Africa and the Middle East [3]. It is the second most popular beverage worldwide that is also a major source of dietary flavonoids. It is also known for its medicinal and health benefits in the eastern traditional systems of medicine since ancient times [4] because of its anti-proliferative, antimutagenic, antioxidant, antibacterial, antiviral, immunoregulatory and chemopreventive properties [5]. A detailed chemical composition of green tea obtained from the systematic literature review is shown in Appendix B (Table B1).

Green tea has three major bioactive compounds that have been shown to affect the inflammatory processes involved in the immunomodulation of transplant rejection. The details of these bioactive molecules and how they affect immunomodulation during transplant rejection are discussed below:

I. **Epigallocatechin Gallate (EGCG)**: The most abundant catechin present in green tea is EGCG, and is considered as one of the most bioactive molecules with strong antioxidant activity. This compound is obtained by formal condensation of gallic acid with the (3R)-hydroxy group of Epigallocatechin (EGC). It represents approximately 59% of all catechins [45]. Among green tea catechins, EGCG is abundant in green tea leaves, and has been shown to exhibit strong health-promoting activity, according to structure activity relationship assessment on EGCG, two close parallel aromatic rings and a third aromatic ring vertical to the two parallel rings may play a key role in the pharmacophore activity. This activity may be associated with the number of –OH groups in the catechin. EGCG is a biologically active compound with known anti-inflammatory, anti-carcinogenic, immune regulatory and free radical-scavenging properties. EGCG can control gene expression by epigenetic modification, which affects the regulation of immune system and enhances the population of regulatory T-cells [46]. EGCG also suppresses the proliferation of autoreactive T cells, reduces the production of pro-inflammatory cytokines, and decreases Th1 and Th17 populations in lymphoid tissues [27].

II. **Epicatechin**: Epicatechin represents approximately 6.4% of the total catechin content. It is a flavan-3-ol, widely distributed in nature and present in large amounts in green tea with effective antioxidant activity. Consumption of epicatechin-rich foods is associated with several health benefits such as modulation of reactive oxygen species production, redox signaling, and pro-inflammatory cascades [47].

III. **Gallic Acid**: Gallic acid (GA) and its derivatives are biologically active compounds, which are widely present in plants,

especially in green tea. Gallic acid is a strong natural antioxidant [48]. It has a wide range of biological activities, including anti-inflammatory, anti-microbial and anti-cancer activities [49].

3.2.2. Summary of green tea on immunomodulation pathways affecting transplant rejection

The effect of EGCG, epicatechin, and GA on the inflammatory processes involved in immunomodulation of transplant rejection is summarized in Table 2. The effect of each of the three compounds of green tea affect the pro- and anti-inflammatory pathways are provided below.

3.2.3. Pro-inflammatory pathways affected by EGCG

(I) **Effect of EGCG on NF-κB**: NF-κB is a transcription factor that mediates the gene expression of several pro-inflammatory cytokines and causes acute rejection during transplantation. EGCG effectively inhibits the binding activity of NF-κB to the DNA in a non-competitive manner that results in a reduced transactivation of NF-κB-driven genes. Transactivation of NF-κB is inhibited by EGCG by down-regulating p53 phosphorylation and IKBα degradation [50]. Additionally, EGCG significantly down-regulates p38 MAPK and ERK 1/2 phosphorylation, which leads to the inhibition of expression of pro-inflammatory mediators that causes tissue rejection via NF-κB and AP-1 transactivation [6,51,52]. Suppressor of cytokine signaling 1 (SOCS1) inhibits NF-κB and thus downregulates the expression of cytokines that causes graft rejection. EGCG induces SOCS1 expression through pro-oxidant pathway [53,54].

(II) **Effect of EGCG on AP-1**: Activator Protein-1 (AP-1), is a transcription factor, whose elevated activity has been shown to cause tissue rejection. EGCG reduces AP-1 mediated genes such as IL-6, MCP-1, IL-1β by inhibiting the phosphorylation of JNK and nuclear expression of JNK and c-Jun. EGCG inhibits AP-1 in a non-competitive manner [55].

(III) **Effect of EGCG on ZAP-70**: In TCR signaling pathway, ZAP-70 acts as a linker for the activation of T cells, phospholipase Cγ1, extracellular signaling-regulated kinase, and MAPK kinase activities in CD3-activated T cells. By binding to ZAP-70 with high affinity, EGCG effectively suppresses ZAP-70 mediated IL-2 release and aids in graft survival [56].

(IV) **Effect of EGCG on STAT1**: STAT1 is a transcription factor, which can associate with STAT4 and mediate Th1 differentiation via JAK2 phosphorylation and ultimately, promote tissue rejection. EGCG is found bind to STAT1 and thus reduce proliferation of Th1 population and modulate graft rejection [57].

(V) **Effect of EGCG on JAK2**: EGCG inhibits the kinase activity of JAK2 in competitive manner, by directly binding to the binding pocket of JAK2 and thus reduces STAT activation that is required for Th1 differentiation during acute rejections [58,59].

(VI) **Effect of EGCG on JNK**: EGCG inhibits JNK phosphorylation and thus reduces JNK mediated cytokines expression during graft rejection. It is considered to inhibit in non-competitive manner due to its antioxidant property [55,60].

(VII) **Effect of EGCG on Tollip**: EGCG increases the expression of Toll interacting protein (Tollip), a strong inhibitor of TLR4 signaling. EGCG induces Tollip through 6-laminin receptor signaling pathway [61,62].

(VIII) **Effect of EGCG on STAT3**: STAT3 is an important transducer that mediates Th17 differentiation during tissue rejections. EGCG effectively inhibits STAT3 by binding competitively to the SH2 domain (Arg-609, key transducer in STAT3) and blocks its phosphorylation [63].

(IX) **Effect of EGCG on IMPDH**: IMPDH, an enzyme that catalyzes the NADP-dependent oxidation of IMP to XMP in de novo synthesis, plays a key role in T-cell activation. EGCG inhibits IMPDH and thus suppresses acute rejections after transplantation [64].

3.2.4. Anti-inflammatory pathways affected by EGCG

(I) **Effect of EGCG on Nrf2-Keap1**: Dissociation of the Kelch-like ECH-associated protein 1 (Keap1) from Keap1-Nrf2 complex is an important regulatory step that could mediate graft survival. EGCG directly binds to Keap1 and release Nrf2 from this complex. EGCG induces Nrf2 with an IC50 of 50 μM in a non-competitive manner [65]. EGCG significantly reduces oxidative stress by enhancing the expression of HO-1 via activation of the Nrf2/HO-1 pathway [66].

(II) **Effect of EGCG on STAT5**: STAT5 is an important regulatory transducer in TNFα pathway that mediates Treg phenotype to promote graft survival. EGCG induces STAT5 activation and favors Treg phenotype thereby promoting graft survival [67].

3.2.5. Pro-inflammatory pathways affected by epicatechin

(I) **Effect of epicatechin on NF-κB**: Epicatechin inhibits NF-κB activation by reducing phosphorylation of IKK (Ser178/180) and IκBα (Ser32). It non-competitively inhibits NF-κB and results in a reduced transactivation of NF-κB-driven genes that causes tissue rejection [47,68].

(II) **Effect of epicatechin on AP-1**: Elevated activity of transcription factor AP-1 has been shown to cause tissue rejection. Epicatechin inhibits of AP-1 activity, thereby reducing the expression of AP-1 mediated genes such as IL-6, MCP-1, IL-1β via inhibiting the phosphorylation of JNK, nuclear expression of JNK and c-Jun [55,69].

(III) **Effect of epicatechin on STAT3**: STAT3 is the main transducer of IL-17, promoting Th17 population during transplantation. Epicatechin effectively inhibits STAT3 phosphorylation by attenuating JAK2 [60].

Table 2
Column 1 specifies the type of inflammatory pathway. Column 2 identifies the specific pathway. Column 3–5 use color codes green, yellow and orange to identify if EGCG, epicatechin, and/or GA, respectively affect that specific pathway.

Inflammatory pathways		EGCG	Epicatechin	GA
Pro-inflammatory	ROS induced inhibition of Nrf pathway	●	●	
	TCR induced IL-2 pathway	●		
	IMPDH pathway	●		
	IL-6 pathway	●		
	IL-12 pathway	●		
	HMGB1/HSP60 pathway	●	●	●
	TNF-α induced IL-17 pathway	●		
Anti-inflammatory	Nrf-induced antioxidant enzymes pathway	●	●	
	TNF-α induced TGF-β pathway	●		

3.2.6. Pro-inflammatory pathways affected by GA

(I) **Effect of GA on NF-κB**: Gallic acid inhibits NF-κB activation by inhibiting IκBα degradation and thus blocking p65 NF-κB translocation into nucleus. Gallic acid non-competitively inhibits NF-κB and results in a reduced transactivation of NF-κB-driven genes that causes tissue rejection [49,70].

(II) **Effect of GA on AP-1**: Activator Protein-1 (AP-1) is a transcription factor whose elevated activity has been shown to cause tissue rejection. GA inhibits AP-1 activity thereby reducing AP-1 mediated genes such as IL-6, MCP-1, IL-1β and thus helps in graft survival [55].

3.3. In silico analysis of green tea effects on immunomodulation of transplant rejection

The three bioactive compounds of green tea, individually and in combination, were tested on the *in silico* models of immunomodulation of transplant rejection. The initial results indicated that, at the dose range used in this study, only EGCG showed a measurable and substantial effect on immunomodulation of transplant rejection (data not shown). Hence, it was decided to focus on performing simulations of the effect of EGCG on the nine inflammatory mechanisms of action governing immunomodulation of transplant rejection.

3.3.1. Effect of EGCG on IL-12 mediated Th1 differentiation

Effect of EGCG was analyzed on IL-12 mediated Th1 differentiation by estimating TNF-α and IFN-γ production in naïve CD4+ T cells and the results are provided below. Under control conditions, the system was assumed to be in an inflammatory state with elevated IL-12 levels and undergoing transplant rejection with no green tea supplementation. Under control conditions, in the absence of EGCG from green tea, the levels of IFN-γ were estimated to be 0.14 nM. EGCG from green tea significantly reduced the amount of IFN-γ in a dose-dependent manner. The levels of IFN-γ were 0.015, 0.008 and 0.005 nM for EGCG levels in 10, 20 and 30 mg/kg dose of green tea, respectively, as shown in Fig. 3A. These results indicate that individually, EGCG from green tea has a significant effect on reducing IFN-γ levels.

As shown in Fig. 3B, EGCG from green tea significantly reduced the amount of TNF-α in a dose-dependent manner. The levels of TNF-α were 0.0007, 0.0004 and 0.0002 nM for EGCG levels in 10, 20 and 30 mg/kg dose of green tea, respectively, as shown in Fig. 3B. These results indicate that individually, EGCG from green tea had a significant effect on reducing TNF-α levels.

3.3.2. Effect of EGCG on IL-6 mediated Th17 differentiation

Effect of EGCG was analyzed on IL-6 mediated Th17 differentiation by estimating IL-17 production in naïve CD4+ T cells and the results are provided below. Under control conditions, in the absence of EGCG from green tea, the levels of IL-17 were estimated to be 0.1919 nM. EGCG from green tea reduced the amount of IL-17 in a dose-dependent manner, however, the reduction was not substantial. The levels of IL-17 were 0.1917, 0.1915 and 0.1913 nM for EGCG levels in 10, 20 and 30 mg/kg dose of green tea, respectively, as shown in Fig. 4.

3.3.3. Effect of EGCG on TNF-α mediated Treg differentiation

Effect of EGCG was analyzed on TNF-α mediated Treg differentiation by estimating the transcriptional factor Foxp3 production in naïve CD4+ T cells. Foxp3 serves as a lineage specification factor of Treg cells and is also associated with expression of TGF-β [71]. The results are provided below.

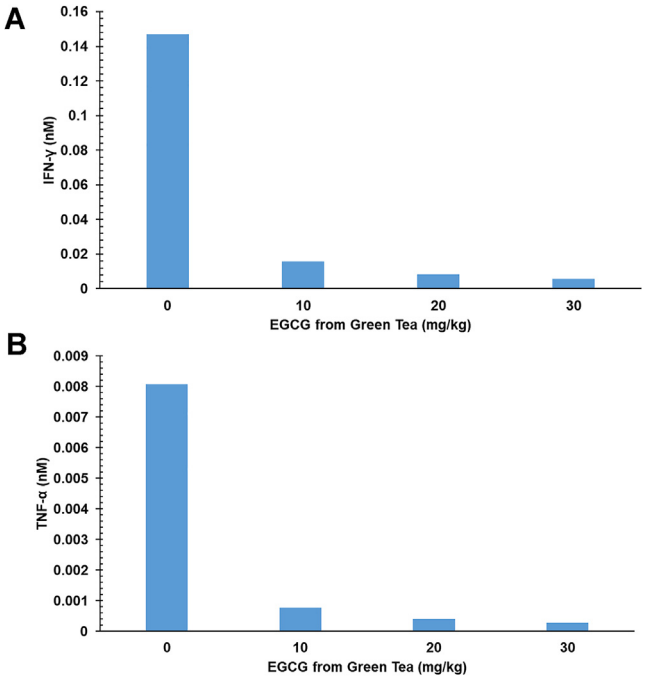


Fig. 3. A: Effect of EGCG from green tea on IL-12 induced IFN-γ in naïve T cells. **B**: Effect of EGCG from green tea on IL-12 induced TNF-α production in naïve T cells.

Under control conditions, in the absence of EGCG, the levels of Foxp3 were estimated to be 2.33E-05 nM. EGCG increased the amount of Foxp3 in a dose-dependent manner. The levels of Foxp3 were 2.82E-05, 3.22E-05 and 3.61E-5 nM for EGCG levels in 10, 20 and 30 mg/kg dose of green tea, respectively, as shown in Fig. 5. These results indicate that individually, EGCG has a relatively moderate effect on increasing Foxp3 levels.

3.3.4. Effect of EGCG on TNF-α mediated Th17 differentiation

The effect of EGCG was analyzed on TNF-α mediated signaling pathways by estimating NO and IL-6 production in naïve CD4+ T cells and the results are provided below. Under control conditions, in the absence of EGCG from green tea, the levels of NO were estimated to be 1028 nM. EGCG from green tea reduced the amount of NO in a dose-dependent manner. The levels of NO were 1020, 1012 and 1004 nM for EGCG levels in 10, 20 and 30 mg/kg dose of green tea, respectively, as shown in Fig. 6A. These results indicate that individually, EGCG has a relatively moderate effect on reducing NO levels.

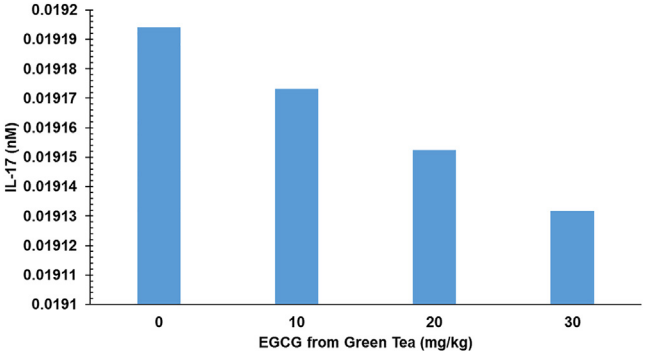


Fig. 4. Effect of EGCG from green tea on IL-6 mediated IL-17 production in naïve T cells.

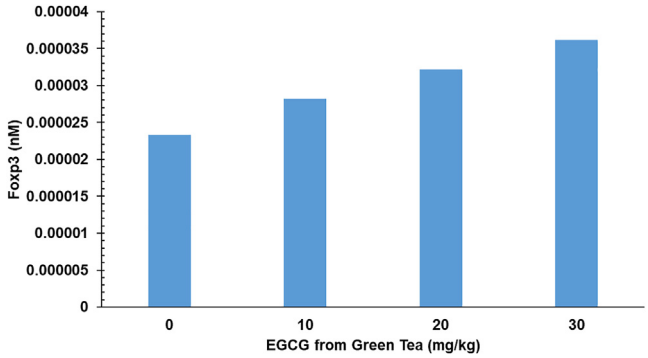


Fig. 5. Effect of EGCG on TNF-α mediated Foxp3 production in naïve T cells.

EGCG reduced the amount of NO in a dose-dependent manner. The levels of IL-6 were 0.0072, 0.00718 and 0.00717 nM for EGCG levels in 10, 20 and 30 mg/kg dose of green tea, respectively, as shown in Fig. 6B. These results indicate that individually, EGCG has a relatively moderate effect on reducing IL-6 levels.

3.3.5. *Effect of EGCG on IL-2 in TCR signaling pathway*

Effect of EGCG was analyzed on TCR signaling pathway by estimating IL-2 production in naïve CD4+ T cells and the results are provided below. Under control conditions, in the absence of EGCG, the levels of IL-2 were estimated to be 0.017 nM. EGCG reduced the amount of IL-2 in a dose-dependent manner. The levels of IL-2 were 0.014, 0.132 and 0.13 nM for EGCG levels in 10, 20 and 30 mg/kg dose of green tea, respectively, as shown in Fig. 7. These results indicate that EGCG has a relatively moderate effect on lowering IL-2 levels.

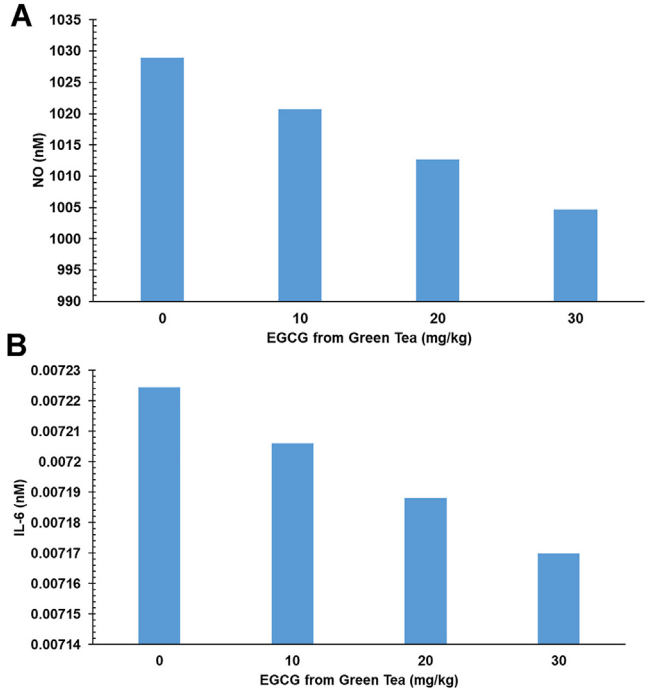


Fig. 6. A: Effect of EGCG on TNF-α mediated NO production in naïve T cells. **B**: Effect of EGCG on TNF-α mediated IL-6 production in naïve T cells.

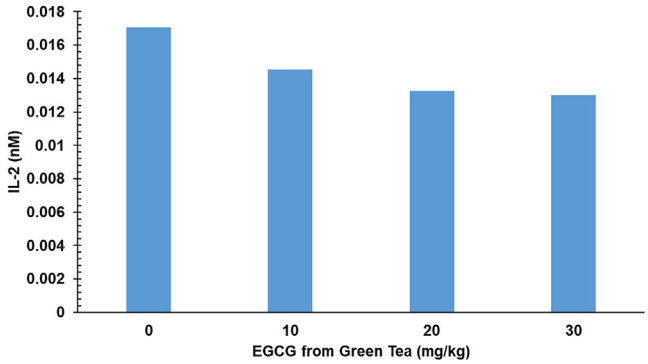


Fig. 7. Effect of EGCG on IL-2 in naïve T cells.

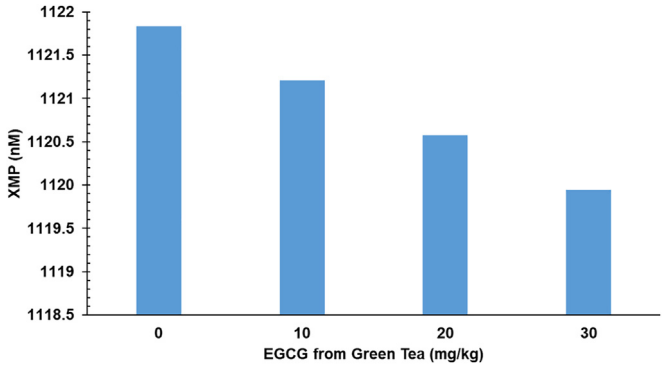


Fig. 8. Effect of EGCG on XMP in naïve T cells.

3.3.6. *Effect of EGCG on IMPDH metabolism*

Effect of EGCG was analyzed on IMPDH Metabolism by estimating XMP production in naïve T cells. IMPDH catalyzed the reaction that converts IMP to XMP, which subsequently initiates de novo synthesis of purines which are essential for cell proliferation [36].

Under control conditions, in the absence of EGCG, the levels of XMP were estimated to be 1121.8 nM. EGCG reduced the amount of XMP in a dose-dependent manner. The levels of XMP were 1121.2, 1120.6 and 1119.9 nM for EGCG levels in 10, 20 and 30 mg/kg dose of green tea, respectively, as shown in Fig. 8. These results indicate that individually, EGCG from green tea has a relatively low effect on lowering XMP levels.

3.3.7. *Effect of EGCG on HMGB1-induced TLR4 signaling pathways*

Effect of EGCG was analyzed on HMGB1-induced TLR4 Signaling pathway by estimating IL-1β, IFN-γ, and TNF-α production in dendritic cells and the results are provided below.

Under control conditions, in the absence of EGCG, the levels of IL-1β were estimated to be 1.38 nM. EGCG reduced the amount of IL-1β in a dose-dependent manner. The levels of IL-1β were 1.29, 1.21 and 1.13 nM for EGCG levels in 10, 20 and 30 mg/kg dose of green tea, respectively, as shown in Fig. 9A. These results indicate that individually, EGCG has a relatively moderate effect on reducing IL-1β levels.

EGCG reduced the amount of IFN-γ in a dose-dependent manner. The levels of IFN-γ were 0.25, 0.23 and 0.22 nM for EGCG levels in 10, 20 and 30 mg/kg dose of green tea, respectively, as shown in Fig. 9B. These results indicate that individually, EGCG has a relatively moderate effect on reducing IFN-γ levels.

EGCG reduced the amount of TNF-α in a dose-dependent manner. The levels of TNF-α were 0.021, 0.02 and 0.019 nM for

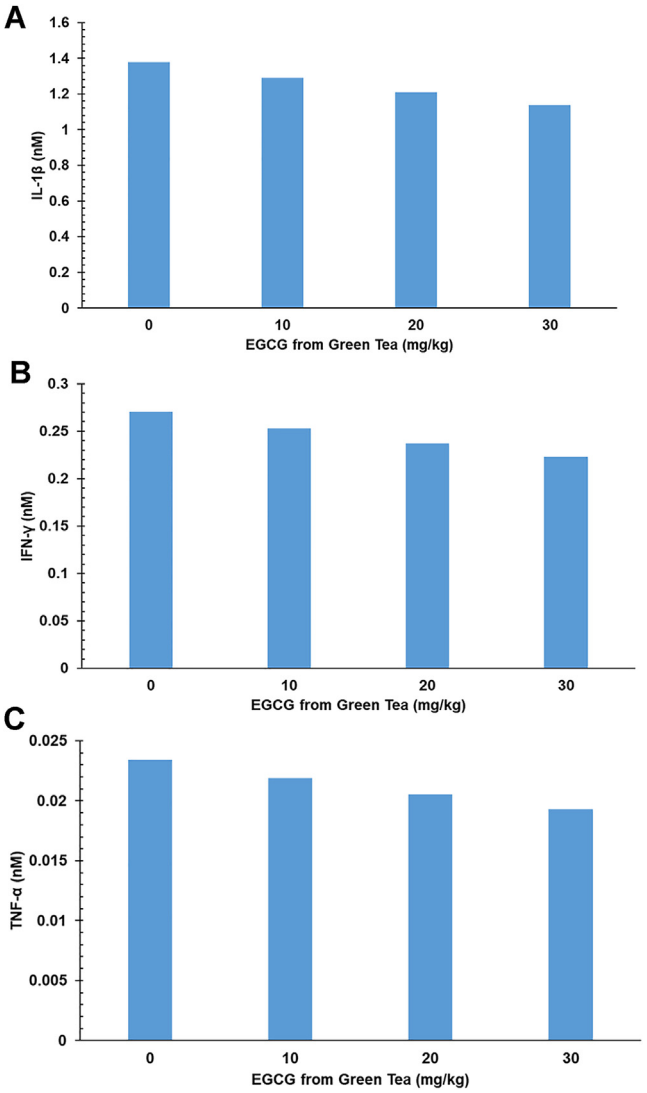


Fig. 9. **A**: Effect of EGCG on IL-1β in dendritic cells. **B**: Effect of EGCG on IFN-γ in dendritic cells. **C**: Effect of EGCG on TNF-α in dendritic cells.

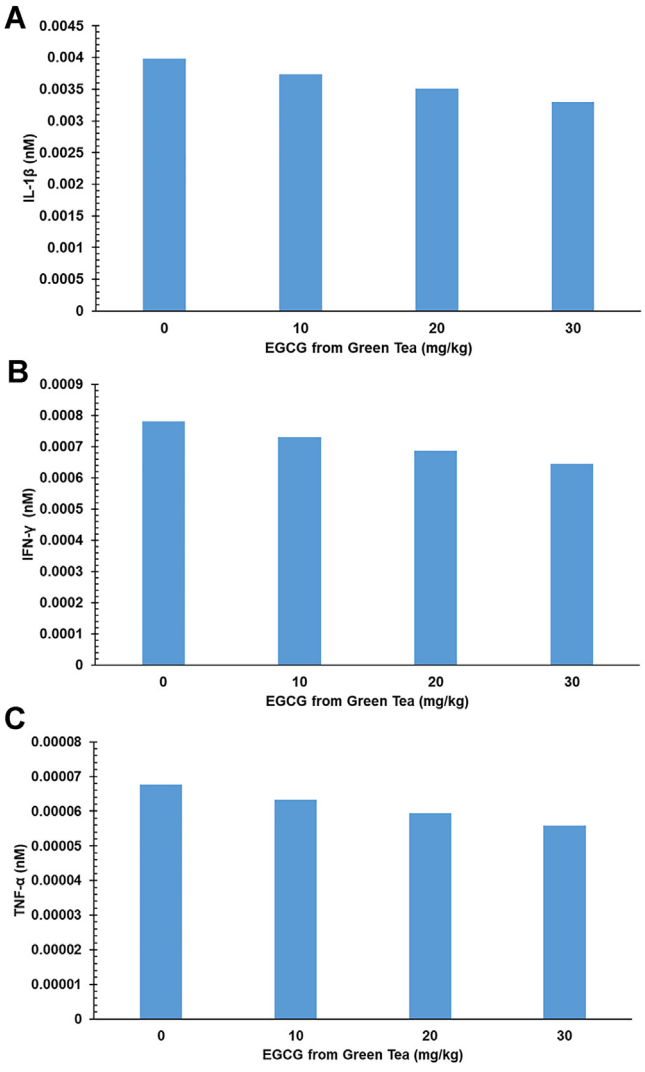


Fig. 10. **A**: Effect of EGCG on IL-1β in dendritic cells. **B**: Effect of EGCG on IFN-γ in dendritic cells. **C**: Effect of EGCG on TNF-α in dendritic cells.

EGCG levels in 10, 20 and 30 mg/kg dose of green tea, respectively, as shown in Fig. 9C. These results indicate that individually, EGCG has a relatively moderate effect on reducing TNF-α levels.

3.3.8. *Effect of EGCG on HSP60-induced TLR4 signaling pathways*

Effect of EGCG was analyzed on HSP60-induced TLR4 Signaling pathway by estimating IL-1β, IFN-γ, and TNF-α production in dendritic cells and the results are provided below. Under control conditions, in the absence of EGCG, the levels of IL-1β were estimated to be 0.004 nM. EGCG reduced the amount of IL-1β in a dose-dependent manner. The levels of IL-1β were 0.0037, 0.0035 and 0.0032 nM for EGCG levels in 10, 20 and 30 mg/kg dose of green tea, respectively, as shown in Fig. 10A. These results indicate that individually, EGCG has a relatively moderate effect on reducing IL-1β levels.

EGCG reduced the amount of IFN-γ in a dose-dependent manner. The levels of IFN-γ were 0.00073, 0.00068 and 0.00064 nM for EGCG levels in 10, 20 and 30 mg/kg dose of green tea, respectively, as shown in Fig. 10B. These results indicate that individually, EGCG has a relatively moderate effect on reducing IFN-γ levels.

EGCG reduced the amount of TNF-α in a dose-dependent manner. The levels of TNF-α were 0.000063, 0.000059 and 0.000055 nM for EGCG levels in 10, 20 and 30 mg/kg dose of green tea, respectively, as shown in Fig. 10C. These results indicate that

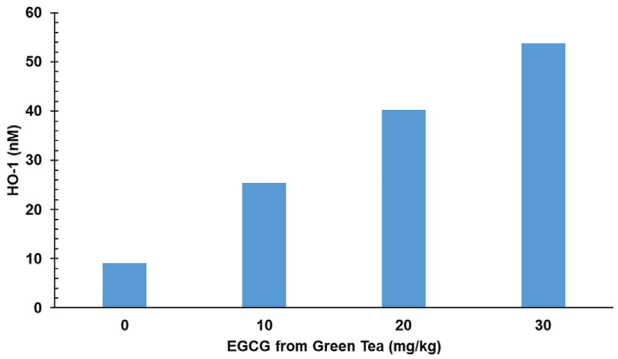


Fig. 11. Effect of EGCG on HO-1 in dendritic cell.

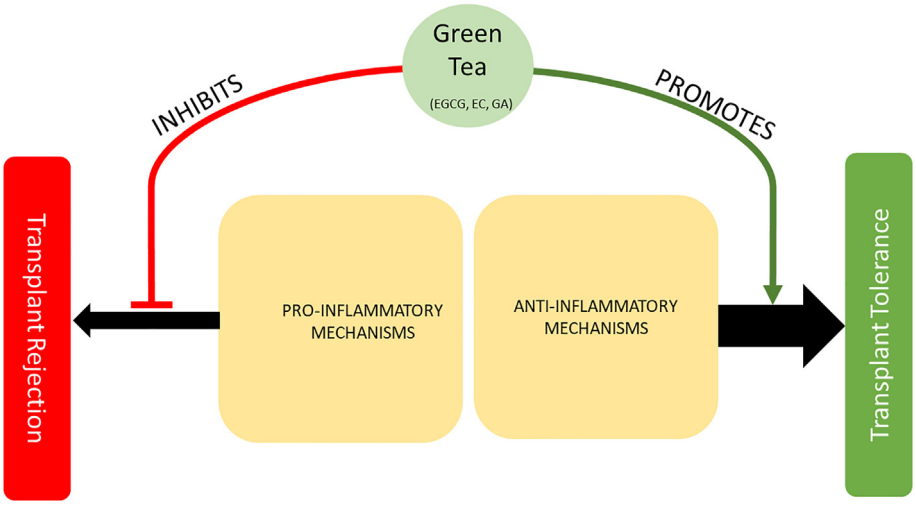


Fig. 12. Effect of green tea on mechanisms of transplant rejection and transplant tolerance.

individually, EGCG has a relatively moderate effect on reducing TNF-α levels.

3.3.9. Effect of EGCG on Nrf2 signaling pathway

Effect of EGCG was analyzed on Nrf2 signaling pathway by estimating HO-1 production in antigen presenting cells and the results are provided below. Under control conditions, in the absence of EGCG, the levels of HO-1 were estimated to be 9 nM. EGCG increased the amount of HO-1 in a dose-dependent manner. The levels of HO-1 were 25, 40 and 43 nM for EGCG levels in 10, 20 and 30 mg/kg dose of green tea, respectively, as shown in Fig. 11. These results indicate that EGCG had a substantial effect on increasing HO-1 levels.

4. Discussion

Systems biology provides a perspective to understand living organisms as being comprised of dynamic networks of biochemical reactions. In this study, a systems level understanding of immunomodulation during transplant rejection emerges from such a systems approach to understand the mechanisms involved in immunomodulation of transplant. The systematic bioinformatics methodology used herein identified nine molecular pathways, across three cell types: naive T cells, CD4+ T cells, dendritic cells, which modulate immune response in transplant rejection. These pathways can be categorized into: 1) two anti-inflammatory pathways, and 2) seven pro-inflammatory pathways. Promotion of Treg and Th2 along with suppression of Th2 and Th17 supports transplant tolerance, while promotion of Th1 and Th17 along with suppression of Treg and Th2 enhances transplant rejection.

A closer review of Fig. 2 reveals that, when it comes to enabling transplant rejection from the seven pro-inflammatory mechanisms, six mechanisms promote Th17 (as traced by the blue lines); four mechanisms promote Th1 (as traced by the red lines); one mechanism suppresses Th2 (as traced by the green line); and, one mechanism suppresses Treg (as traced by the purple line). Similarly, a closer review of the two anti-inflammatory mechanisms reveals, when it comes to enabling transplant tolerance from the two anti-inflammatory mechanisms, two mechanisms promote Treg (as traced by the purple lines); one mechanism promotes Th2 (as traced by the green line); and, one mechanism suppresses Th1 (as traced by the red lines). The architecture provides the opportunity for a holistic molecular systems understanding of the role of inflammation in immunomodulation of transplant rejection that has heretofore not previously existed, to the best of our knowledge.

There is a growing body of in vitro, *in vivo* and clinical evidence demonstrating the anti-inflammatory and antioxidant properties of green tea [46,72,73]. Green tea has been used as a medicine in the traditional systems of medicine for many millennia [3,4]. The systems biology approach herein identifies bioactive compounds of green tea and the mechanisms by which those compounds may affect the molecular mechanisms of immunomodulation post-transplant. The results show that the bioactive compounds from green tea affect transplant tolerance and transplant rejection by inhibiting immune response from pro-inflammatory Th1 and Th17 cells, and promoting immune tolerance by increasing anti-inflammatory Treg and Th2 cells proliferation, respectively. The results from this study indicate that EGCG, of all the three bioactive compounds in green tea, affects both pro- and anti-inflammatory pathways to beneficially enhance transplant tolerance. The overall effect of green tea on immunomodulation during transplantation is summarized in Fig. 12.

The computational systems biology analysis reveals mechanisms of action for a potential intervention for enhancing immune tolerance in the clinical setting. Our results show that, of the three green tea bioactive compounds, only EGCG had a significant impact on the immunomodulatory pathways by: 1) suppressing the immune response from pro-inflammatory Th1 and Th17 cells; and, 2) promoting immune tolerance by increasing anti-inflammatory Treg cells proliferation.

Among the pro-inflammatory pathways, EGCG substantially lowered IFN-γ and TNF-α production via IL-12 signaling in naïve T cells. IL-12 creates an inflammatory environment during transplantation through IFN-γ and TNF-α which favors Th1 differentiation and subsequent immune rejection of the graft by Th1 cells [26,27]. Similarly, EGCG was found to be effective in suppressing the inflammatory environment by reducing IL-2 production via TCR signaling. IL-2 is a potent pro-proliferative cytokine that expands the T clone itself and strengthens the adaptive response against the graft [34,35]. At the tested dose levels, EGCG exerted a lower effect on the remaining pro-inflammatory pathways such as TNF-α signaling, TLR4 signaling and IMPDH metabolism. However, EGCG significantly upregulated the antioxidant enzyme HO-1. The antioxidant system plays a key role in mitigating ischemia reperfusion injury, a major cause of transplant rejection [74]. EGCG also promoted Treg phenotype, and hence immune tolerance via increased

production of Foxp3. Increased Foxp3 leads to increased expression TGF-β and IL-10 and overexpression of these cytokines promotes Treg differentiation and immune suppression [26,27,43].

5. Conclusions and future directions

This study, to the best of our knowledge, provides the first molecular mechanistic framework of the potential value of incorporating green tea, and specifically the bioactive compound EGCG, in clinical nutrition to enable transplant tolerance. The framework developed in this study provides an infrastructure for future analysis and discovery of individual and/or synergistic combinations of either pharmaceutical or natural compounds on immunomodulation in transplant rejection.

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Author contributions

Shiva Ayyadurai: Conception and design of the study, analysis and interpretation of data, drafting the article or revising it critically for important intellectual content, final approval of the version to be submitted. **Prabhakar Deonikar:** Design of the study, acquisition of data, analysis and interpretation of data, drafting the article or revising it critically for important intellectual content, final approval of the version to be submitted.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnesp.2021.09.012.

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