Genomics of FOXP3 Enhance Treg and Maintain Immune-homeostasis

Shouhartha Choudhury (shouharthac@gmail.com)
Assam University

Research Article

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Abstract

The immunologic theorem is a subject of self also non-self differentiation directly combat infections and maintains the energy of self-antigens. Immune privilege of T-cells is prevailing via crucial and superficial prospect. The T-cell promotes the function of immune replication and implant antigens. The regulatory T-cells (Treg) impart the progress of diseases also prevent immunity. X chromosome encoded FOXP3 is a regulator of segregation and immunosuppressive function. Nuclear factor FOXP3 regulated lineage-specific polarity of Treg crucially maintains immune-homeostasis. The functional inhibitions of helper T cells by FOXP3-inhibitory peptide constitute an imprint to enhance immunotherapy. The enlightenment of FOXP3 contributed to a novel concern in the experiment of suppressor T lymphocytes and mechanisms of immune homeostasis. In this exploration, I aimed to view FOXP3 functions from the FOX family in Homo sapiens and compare them with Mus musculus. Therefore, I performed a bioinformatics pipeline for the experimentation of the forkhead box P3 and their family. My finding data supported the FOX family of TF's play a preventive strategy during the development. A ray of FOXP3 enhances Treg and maintains immunity against infections. The specific bioinformatics analysis epitomized FOXP3 as a T-cell dependent gene that can help to interpret the outcome in infection biology.

Highlights:

- The present finding epitomized the FOXP3 is a T-cell dependent gene.
- FOXP3 enhances CD4+ cells to express the cytotoxic T cell (CD8+ cells).
- Since the T-cell dependent gene can help to protect immunity against infections.

Introduction:

The genomic era is a feature of the rapid examination of particular gene fragments essential for cellular responses. Following infectious diseases i.e. bacteria, viruses, fungi, parasites also tumour represents a substantial but resistant part of our genome that can control infections. Our immune system can holds infections aside, maintain infected tissues and refuse immune-pathologic damage. The immunologic impact achieves by the responses of our genome that interprets the immune process. The result is dynamic, but few differences between the genome and host cells can jeopardize, adaptive and cooperative. This hypothesis emerged leading questions in biology: how we specify diseases, illuminate genome sequencing study and control infections [1, 2]. The postulation of T-cells is the main factor of protective immunity against infections & their function design active protection. Immunological reviews supported a complex differentiation process appears during CD8 + T-cell reaction in various illness. The cells responses secure under T-cells adaptation between three predictable stages: (a) primary infection and proliferation, (b) termination stage and (c) initiation of T-cells. These phases of T-cell response protected by complex transcription and functions change the results, expand and gain effectors functions, and survive through the termination stages. This study will explain the average intellect of how functional and protective CD8 + T-cell responses generate and maintain various infections. The theorem
of infections divided into two types: (1) acute infections and (2) chronic infections. Acute infections commonly result from active immune reactions and chronic infections associated with suboptimal T-cell function. In this object, my focus on different infections and knowledge of the CD8+ T-cell polarity appears when the antigen eliminates via prime infection and how T-cell reactions can change and reduce chronic infections. The chronic infection effected by cytotoxic T-cells (CD8+ T-cell) of the immune process. The CD8+ T-cell reactions steadily reduce infections then cells gradually evacuate function during chronic illness. These processes enhance by helper CD4+ T-cells that control the infection and contribute a reaction of CD8+ T-cells [3, 4]. Those concepts in the immune process raise a question, how and why CD4+ T-cells enhance CD8+ T-cells reaction [5–7]? An immunogenic function of cytokines generates by CD4+ T-cells is well-known as interleukin-21 (IL-21) that governs chronic infections. The IL-21 promotes by the process of different CD4+ T-cells subgroup i.e. TFH, extended T-helper 17 cells and LGL [8, 9]. The cytokine promotes juxtapose with these subgroups and help other immune cells like large granular lymphocytes (LGL), B-cells and cytotoxic T cell for a proper response of IL-21. The IL-21R element of the gamma chain family of cytokine receptor included IL-2 (CD25), IL-7, IL-15, and IL-21 combine to improve CD8+ T-cell reactions [10–12]. Those groups compare to CD4+ T-cell functions for CD8+ T-cell response are associated with IL-21 as an intermediator. During this activity, the newly synthesized proteins will reduce peptide fragments even infected cells lose cytotoxic granules. The stimulation of CD8+ T-cells produces clear lymph nodes, whereas antigen appearance cells and naive T-cell interact with one another. In the lymph node, DC and CD4+ T-cells contribute to the co-stimulation is vital for confirmation of the tendency of CD8+ T-cells. Furthermore, the initial face of chronic and acute infections, Th1, Th2 and Th17, CD8+, CD4+ cells negotiate preventive immunity against illness [13, 14]. The immune reaction moderate by regulatory cells like Treg, M2-type macrophages, MDSCs, TGF-β, cytokines and IL-10 innate adaptive response in immune-checkpoints that control T-cell activation. These regulatory cells in immune checkpoints enhance infections as a mechanism of immune breakthrough and become unique targets for treatment. However, the hypothesis of FOXP3 functions in Treg cells linked with MHC2 that control CD4+ T-cells also expresses a high equilibrium of IL-2 (CD25). An addition of FOXP3 function in CD4+ T-cells and IL2 appears by the process of MHC1 governs CD8+ T-cells reactions. The CD8+ T-cells can kill pathogens by reactions of perforin and granzymes, which disrupt the plasma membranes of infected cells. Also, Treg included Tr1, Th cells, CD8, CD28 and HLA-E control T-cells. The Treg activity associated with CTLA-4 and CD28 receptor potentially bound with two natural ligands such as B7-1 and B7-2 effective for Treg-suppressive capacity [15–17]. The CD86 sharply enhance suppression by the exercise of CD25+ and CD4+ cells. Invariance, resistance mechanisms of CD80 enhances proliferative response by reducing Treg suppression. The combined effect of B7 and B7.2 on DC regulates for improvement of unstable to a stable state with the potentiality of Treg-suppressive response. The B7-2 and B7-1 emulate functions via CD152 and CD28 on Treg that has a remarkable outcome on the repression of immune reaction. The tendency of Th cells in immune checkpoints included CTLA-4, PD-1 (CD279), TIM-3, LAG-3 and TIGIT. Furthermore, the significant function of PD-1 mainly expresses on T-cells that negotiate with their two ligands PD-L1 (B7-H1, CD274) and PD-L2 (B7-DC, CD273) rival a unique role of initiation and maintain peripheral tolerance mechanism. The PD-L1 and PD-L2 express APCs and infected cells release a negative signal to T-cells are called T-cell exhaustion.
Therefore, antibodies binding CTLA-4, PD-1 or PD-L1 have a dynamic efficiency to enhance immunotherapy \([18–23]\). The defensive mechanisms suggested the X-chromosome encoded FOXP3 transcription factor gene govern T-cells polarity and immunosuppressive function. The Treg express from FOXP3 and control immune-mediated infections. The nuclear TF of FOXP3 regulates lineage-specific conflict of the Treg that certainly a defence of the immune-homeostasis \([12]\). The FOXP3 function supported as a lineage associated factor in the regulatory T-cells. The activity of Treg due to loss of FOXP3 function is vigorous and untreatable immune-mediated infections. The functional inhibition of Treg by forkhead domain associated peptides initiate an impact to promote immunotherapy \([24–26]\). Recent studies supported the FOXP3 function attain unique mechanisms in the biology of Treg and cellular-immune-homeostasis. Those reports revealed that the study of FOXP3 functions and immunologic mechanisms involved with regulatory T-cells. In this work, intense evidence of the FOX family of TF's reveals a leading function during the growth of organisms. In contrast, the X chromosome encodes FOXP3 enhances Treg and improve immunity against infections.

**Results:**

**Structural analysis of target gene:**

The target sequence determined the building block of nucleotides and peptides. The predominant sequence formulated of 1296 nucleotides and 431 peptides with 83 peptides residue binding to DNA is well-known as a forkhead domain (Table 1). The polypeptide structure demonstrated the forkhead domain is involved in DNA binding. The forkhead domain is also known as the “winged helix” domain. The analysis of three-dimensional structure determined alpha-helices also beta-sheets link up within B-DNA as a monomer interconnects with the DNA backbone. The alpha helices pretend a thick structure that extends the third helix to the major groove. The polypeptides compose a warped anti-parallel beta-sheet and stochastic coil linked with the minor groove (Fig. 1).

**Genome-wide analysis:**

The genome-wide analysis by the HMMER algorithm obtains 113, 87 of forkhead domains in Homo sapiens and Mus musculus, respectively (Table: 2). The BLAST2 results represent 115, 87 of the homologs FOXP3 gene in Homo sapiens and Mus musculus, respectively (Table: 2). Further analysis of Gene Ontology Annotation confirmed the sequence accuracy of FOXP3 in two different organisms (Table: 3). These standalone searches concluded the FOXP3 was present in both organisms.

**Domain, motifs and chromosome location analysis:**

The highest hits of target (FOXP3) gene selected from Homo sapiens and Mus musculus for MSA, a multiple sequence alignment (MSA) determined the conserved forkhead domain in the two different organisms. The high consensus (90%) sequence indicated the extended FOX domain and its specific
motifs (Fig. 2 & 3). The chromosome localization study confirmed that the FOXP3 located band Xp11.23, start 49,250,436 bp also end 49,270,477 bp (Fig. 4).

Gene expression and network analysis:

The disease state study suggested the up-regulated expression of FOXP3 in 21 of COVID-19 clinical cohorts correlated in mild/asymptomatic and mild/normal blood samples. Also, the down-regulated expression of FOXP3 in 21 COVID-19 patients correlated in asymptomatic/normal blood samples. The anatomical segments of the data of human mRNA showed the medium level FOXP3 express in tissue, circulatory system, and blood (Fig. 5). The gene network analysis determined the FOXP3 interacts with other molecules. Such as runt-related transcription factor 1, TNFRSF18 (CD357), RAR-related orphan receptor gamma, CTLA-4 (CD152), GATA binding protein 3, Interleukin-2, Interleukin-2 receptor alpha chain, Interferon-gamma (IFNγ), NFATC2, those molecular interactions govern the fundamental function of FOXP3 in the cellular process (Fig. 6).

Discussion:

The defensive immune responses against infections depend on the T-cells that can control and destroy infected cells. Activation of T-cells determined by antigen-presenting cells i.e. DCs (dendritic cells), macrophages and B & T lymphocytes [27–29]. In this study, I discuss the prospective peptide-based targets for the protection and elimination of infections. I justify improvement that governs protective element with quality of antigen, supplement and foundation. Also, I will elucidate adaptive peptide base protection against infectious. The leading aim of this work is the examination of peptide inhibitors protein to overcome immunosuppressive response against infections. Those infection diseases such as bacteria, viruses, fungi, parasites and tumours are the origin of acute and chronic infections. Severe infections such as a common cold, cough and fever are usually clear from patients within a week. These types of infections do not cause any risk factor. However, acute and chronic illness originated from the burden of pathogenesis. Chronic infections are persistent that causes by the inefficient immune response lead to long-lasting symptoms. Following the annual pandemic results in about 3–5 million patients affected and nearly 250,000–500,000 deaths worldwide [30–35]. Acute and chronic illness causes major health impact in human because infections have a collision on health strategies to limit or control infections. According to genome sequencing, mammals evolved a refine-immune system to cope with all kind of illness. Precisely, the acquired immune system is most important to control various infections. The immune reactions embrace specific antibodies against infections that can interrupt and restrain pathogenesis. Therefore, a notable breakthrough in this work is the function and mechanisms of regulatory T-cells (Tregs) are a clear lymphocyte lineage endowed with inhibitory properties that effected catalytic reaction in the immune system. Treg (CD4 + cells) described by the function of FOXP3 (specific forkhead box/winged-helix transcription factor 3), which is vital for the growth of organisms [36–38]. In this work, my finding suggested the peptide inhibitor can control the efficacy of immunity because the signal of the conserved forkhead domain appears inside the cell. Most prominently, the migration of
FOXP3 nuclear translocation probably inhibits the adaptability of Treg. That process ensues FOXP3 ability to recover proliferation and enhance Treg. The FOXP3 being a homo-oligomeric element with paramount molecular substance, inconsistent polypeptides interact with different molecules such as RUNX1, TNFSF18, CTLA4, GATA-3, RORC, NFATC2, IL2, IL2RA, IFNG and IL10. So, blocking interactions between IL-10R and PD-L1 outcome of infections eliminated [39–46]. It logically assumed that the interactions of peptides and other molecules govern the ultimate function of FOXP3. Also, the interruption of participants might decline the innate reaction. The combined concepts of immunologic and molecular mechanisms contributed primary evidence of suppressive functions of FOXP3-dependent-Treg are vital for immune-homeostasis [12]. Therefore, Treg activity is dynamic for defence against various infections. Those mechanisms illustrated the elevated number of specific CD4 + T-cells or Treg cells infected by pathogens. The peptide-base targets should elicit a robust T-cell renewal since the T-cells require reacting immediately after infections to terminate the pathogens before it causes illness or disease burden. That protective strategy evokes responses required specific translocation is valuable. The broad and specific T-cell reaction can control infections because T-cell response should be productive against infections without harms. The peptide-based targets can formulate antibodies also T-cell response since the peptides are synthetic, safe and secure to produce [47]. Difficult to prescribe accurate targets also prefer leading molecules that improve immunity and drive immune reactions in conscious treatment.

**Conclusion Remarks:**

The present finding concluded the FOX family play dominant roles during the development of organisms. The ray of the FOXP3 gene identified as a T-cell dependent gene is an effective target for preventive strategy in mammals since the major T-cell dependent gene can define the real spectrum of infections. Besides, the fusion genes or genetic recombination may be an earlier marker for research and development. The specific fusion genes or genetic recombination may be immunogenic and efficient in inducing IgG antibody. Therefore, the statement of pathogenesis raises a step for new and exciting experiments mandatory to explore the feature of our biology.

**Materials And Methods:**

**Query sequence and database**

Target sequence retrieved from the various species-specific databases (NCBI, UniProt, KEGG, GenBank, EMBL, and DDBJ) and performed web-based application SMART for identification of particular domain in the target sequence. SWISS-MODEL is a bioinformatics web-server for comparative modelling of three-dimensional structures. This application performed for generating a 3D structure. That application routinely used in many practical applications. The SWISS-MODEL is an updated database of comparative and 3D model of organism proteome for biomedical research.
The draft genome sequence downloaded from genomic data in various specialized databases (Ensemble and NCBI).

**Standalone tools**

HMMER executes multiple sequence alignments of the specific domain as a profile search. HMMER is a statistical algorithm that offers to make MSA of the particular domain as a profile search is a probabilistic model called the profile HMM. Standalone BLAST performs for homologs gene in organisms.

**Annotation**

The BLAST2GO performed for gene annotation is a bioinformatics and computational tool for high-throughput gene ontology annotation. The functional statistics of genes recompense via GO annotation is a glossary of the working attribute.

**Domain**

For examination of sustain domain in sequences. The MSA method is applicable to reckon the highest match of homologs sequences and then observe similarity, identity and differences. MSA of maximum hits of target sequences examination by web-based tool MultAlin for validation of sustain domain.

**Motif**

The MEME tool performs for examination of sequence-specific motifs. MEME suite is a bioinformatics and computational web base tool to discover and validation of the sequence-specific motifs.

**Chromosome location**

The chromosome location rectified using the gene card. The gene card is a database of human provides statistics of all predicted human genes. That database is presently available for medical research like gene, protein and associated disease.

**Gene networks**

The genetic network is a cluster of molecules that regulate and interact with each other in the cells to command the development levels of proteins or mRNA. Some protein serves to activate genes are TF’s that bind to the pioneer area and initiate the activity of other proteins is called regulatory cascades.
retrieve the STRING database for the prediction of protein-protein interaction. The STRING database contains various resources like experimental data and computational methods.

**Abbreviations**

CTL: Cytotoxic Lymphocyte

APC: Antigen Presenting Cell

MHC1: Major Histocompatibility Class 1

MHC2: Major Histocompatibility Class 2

CD8+ T-cell: Cytotoxic T-cell

DCs: Dendritic cells

NK: Natural Killer

IgG: Immunoglobulin G

CD4+ Cells: T helper cells

CD80 (B7-1): Cluster of differentiation 80

CD86 (B7-2): Cluster of Differentiation 86

B7-DC (CD273): Programmed cell death 1 ligand 2

B7-H1 (CD274): Programmed death-ligand 1

PD1 (CD279): Programmed cell death protein 1

CD28: Cluster of Differentiation 28

CTLA-4 (CD152): Cytotoxic T-lymphocyte-associated protein 4

DNA: Deoxyribonucleic acid

NCBI: National Center for Biotechnology Information

KEGG: Kyoto Encyclopedia of Genes and Genome

SMART: Simple Modular Architecture Research Tool

EMBL: European Molecular Biology Laboratory
BLAST: Basic Local Alignment Search Tool

HMM: Hidden Markov Model

GO: Gene Ontology

MEME: Multiple EM for Motif Elicitation

TF’s: Transcription factors

MDSCs: myeloid-derive suppressor cells

Treg: regulatory T cells

HLA-E: MHC class I antigen E

MHC1: MHC class I

MHC2: MHC Class II

MHC: Major Histocompatibility Complex

IL2: Interleukin 2

MDSCs: Myeloid-derived suppressor cells

TGF-β: Transforming growth factor beta

IL-10: Interleukin 10

Th1: T helper type 1

Th2: T helper type 2

Th17: T helper 17

IL-7: Interleukin 7

IL-15: Interleukin 15

IL-21: Interleukin 21

T_{FH}: T follicular helper cells

TIM-3: T-cell immunoglobulin and mucin-domain containing-3

LAG-3: Lymphocyte-activation gene 3
Declarations

Ethical approval and consent to participate:

Consent for publication:

The work furnished in this paper is original and communicated by the correspondent given in the manuscript. The author disclosed that the document is not concern elsewhere and not receive for evaluation by other journals.

Ethical approval:

The study contains an in-silico analysis of the mammalian genome to examine the particular gene in different organisms.

Availability of data and material:

The data and materials have not been deposited yet in the database. The HMMER, BLAST2, and BLAST2GO (gene ontology annotation) data are available by request or demand.

Competing interest:

The author stated that the work has no conflict of interest.

Funding:

The author did not avail of financial assistance from any source in undertaking the present study.

Author’s contributions:
This research paper contains an author placed at the top of the manuscript. The author conceived the idea, experimented, analyzed data and also prepared the manuscript.

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References

1. Herbert W Virgin, E John Wherry, and Rafi Ahmed. Redefining chronic viral infection. Cell. July 2009; 138 (1):30–50
2. Ahmed. R and Gray D. (1996). Immunological memory and protective immunity: understanding their relation. Science. April 1996; 272 (5258):54–60
3. H. Shin, E. J. Wherry. CD8 T cell dysfunction during chronic viral infection. Curr. Opin Immunol. Aug 2007;19(4):408–415
4. M. A. Williams, M. J. Bevan. Effector and memory CTL differentiation. Annu. Rev. Immunol. April 2007; 25:171–92
5. H. Elsaesser, K. Sauer, D. G. Brooks. IL-21 is required to control chronic viral infection. Science. Jun 2009; 324(5934):1569–1572
6. J. S. Yi, M. Du, A. J. Zajac. A vital role for interleukin-21 in the control of a chronic viral infection. Science. Jun 2009; 324(5934):1572–1576
7. A. Fröhlich. IL-21R on T cells is critical for sustained functionality and control of chronic viral infection. Science. Jun 2009; 324(5934):1576–1580
8. R. Spolski, W. J. Leonard. (2008). Interleukin-21: basic biology and implications for cancer and autoimmunity. Annu. Rev. Immunol. April 2008; 26:57–79.
9. J. S. Silver, C. A. Hunter. With a little help from their friends: interleukin-21, T cells, and B cells. Immunity. July 2008; 29(1):7–9
10. Rong Zeng, Rosanne Spolski, Steven E. Finkelstein, SangKon Oh, Panu E. Kovanen, Christian S. Hinrichs, Cynthia A. Pise-Masison,Michael F. Radonovich, John N. Brady, Nicholas P Restifo, Jay A. Berzofsky, and Warren J. Leonard. Synergy of IL-21 and IL-15 in regulating CD8 + T cell expansion and function. Exp. Med. Jan 2005; 201(1):139–148
11. M. T. Kasaian. IL-21 limits NK cell responses and promotes antigen-specific T cell activation: a mediator of the transition from innate to adaptive immunity. Immunity. April 2002; 16(4):559–569
12. Alexander Y. Rudensky. Regulatory T Cells and Foxp3. Immunol Rev. May 2011; 241(1):260–268
13. Lydia Dyck and Kingston H.G. Mills. Immune checkpoints and their inhibition in cancer and infectious diseases. European Journal of Immunology, May 2017; 47(5):765–779
14. Yong Zheng, Claire N. Manzotti, Michael Liu, Fiona Burke, Karen I. Mead and David M. Sansom. CD86 and CD80 Differentially Modulate the Suppressive Function of Human Regulatory T Cells. J Immunol Mar 2004; 172:2778–2784

15. Redouane Rouas, Hussein Fayyad-Kazan, Nabil El Zein, Philippe Lewalle, Franc-oise Rothe, Alexandru Simion, Haidar Akl, Mohamad Mourtada, Mohamad El Rifai, Arsene Burny, Pedro Romero, Philippe Martiat and Bassam Badran. Human natural Treg microRNA signature: Role of microRNA-31 and microRNA-21 in FOXP3 expression. Eur. J. Immunol. Jun 2009; 39:1608–1618

16. Macfarlane Burnet. Auto-immune Disease II. Pathology of the Immune Response. Br Med J. Oct 1959; 2:720–725

17. Yasumasa Ishida, Yasutoshi Agata, Keiichi Shibahara and Tasuku Honjo. Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. EMBO J. Nov 1992; 11:3887–3895

18. Tyler R. Simpson, Fubin Li, Welby Montalvo-Ortiz, Manuel A. Sepulveda, Katharina Bergerhoff, Frederick Arce, Claire Roddie, Jake Y. Henry, Hideo Yagita, Jedd D. Wolchok, Karl S. Peggs, Jeffrey V. Ravetch, James P. Allison, and Sergio A. Quezada. Fc-dependent depletion of tumor-infiltrating regulatory T cells co-defines the efficacy of anti-CTLA-4 therapy against melanoma. J Exp Med. Aug 2013; 210:1695–1710

19. Shohei Hori, Takashi Nomura and Shimon Sakaguchi. Control of Regulatory T Cell Development by the Transcription Factor Foxp3. Science. Feb 2003; 299(5609):1057–1061

20. Yoshihiro Ohue and Hiroyoshi Nishikawa. Regulatory T (Treg) cells in cancer: Can Treg cells be a new therapeutic target? Cancer Sci. Jul 2019; 110(7):2080–2089

21. Nicholas L Syn, Michele W L Teng, Tony S K Mok, Ross A Soo. De-novo and acquired resistance to immune checkpoint targeting. The Lancet Oncology. December 2017; 18(12): 731–741

22. Peter C. Doherty, Barbara B. Knowles, and Peter J. Wettstein. Immunological Surveillance of Tumors in the Context of Major Histocompatibility Complex Restriction of T Cell Function. Advance in Cancer Research. 1984; 42:1–65

23. Madhav V. Dhodapkar and Ralph M. Steinman. Antigen-bearing immature dendritic cells induce peptide-specific CD8 + regulatory T cells in vivo in humans. July 2002; 100:174–177

24. Noelia Casares. A Peptide Inhibitor of FOXP3 Impairs Regulatory T Cell Activity and Improves Vaccine Efficacy in Mice. J Immunol. Nov 2010; 185(9):5150–5159

25. Lisa D. S. Johnson and Stephen C. Jameson. A Chronic Need for IL-21. Science. Jun 2019; 324(5934):1525–1526

26. Jason D. Fontenot, Marc A. Gavin and Alexander Y. Rudensky. Foxp3 programs the development and function of CD4 + CD25 + regulatory T cells. Nature Immunology. Apr 2003;4(4):330–336

27. Ira Mellman and Ralph M. Steinman. Specialized and Regulated Antigen Processing Machines. Cell. Aug 2001;106(3):255–258
28. S C Knight, R Hunt, C Dore, and P B Medawar. Influence of dendritic cells on tumor growth. PNAS. July 1985; 82(13):4495–4497

29. David M. Richards, Jan Hettinger, and Markus Feurerer. Monocytes and Macrophages in Cancer: Development and Functions. Cancer Microenvironment. Aug 2013;6(2):179–191

30. Bruno Lemaitre, Jean-Marc Reichhart, and Jules A. Hoffmann. Drosophila host defense: Differential induction of antimicrobial peptide genes after infection by various classes of microorganisms. PNAS. Dec 1997;94 (26):14614–14619

31. Sietske Rosendahl Huber, Josine van Beek, Jørgen de Jonge, Willem Luytjes and Debbie van Baarle. T cell responses to viral infections opportunities for peptide vaccination. Immunology. Apr 2014; 5:171

32. Knipe DM, Howley PM. Fields Virology. Philadelphia: Lippincott Williams & Wilkins. 2013

33. Lycke E, Hamark B, Johansson M, Krotochwil A, Lycke J, Svennerholm B. Herpes simplex virus infection of the human sensory neuron. An electron microscopy study. Arch Virology. 1988; 101(1–2):87–104

34. WHO. Influenza Fact sheet 2009

35. Zhao Y, Zhang YH, Denney L, Young D, Powell TJ, Peng YC. (2012). High levels of virus-specific CD4 + T cells predict severe pandemic influenza A virus infection. Am J Respir Crit Care Med. Dec 2012;186(12):1292–7

36. Wan, Y. Y., and R. A. Flavell. Regulatory T-cell functions are subverted and converted owing to attenuated Foxp3 expression. Nature. Feb 2007; 445(7129):766–770

37. Williams, L. M., and A. Y. Rudensky. Maintenance of the Foxp3-dependent developmental program in mature regulatory T cells requires continued expression of Foxp3. Nat. Immunol. Mar 2007; 8(3):277–284.

38. Bettelli, E., M. Dastrange, and M. Oukka. Foxp3 interacts with nuclear factor of activated T cells and NF-kappa B to repress cytokine gene expression and effector functions of T helper cells. Proc. Natl. Acad. Sci. USA. Apr 2005; 102(14):5138–5143.

39. Bin Li, Arabinda Samanta, Xiaomin Song, Kathryn T. Iacono, Patrick Brennan, Talal A.Chatila, Giovanna Roncador, Alison H. Banham, James L. Riley, Qiang Wang, Yuan Shen, Sandra J. Saouaf and Mark I. Greene. 2007. FOXP3 is a homo-oligomer and a component of a supramolecular regulatory complex disabled in the human XLAAD/IPEX autoimmune disease. Int. Immunol. Jul 2007; 19(7):825–835.

40. Ono, M., H. Yaguchi, N. Ohkura, I. Kitabayashi, Y. Nagamura, T. Nomura,Y. Miyachi, T. Tsukada, and S. Sakaguchi. Foxp3 controls regulatory T-cell function by interacting with AML1/Runx1. Nature. Apr 2007; 4469(7136):685–689.

41. Du, J., C. Huang, B. Zhou, and S. F. Ziegler. 2008. Isoform-specific inhibition of ROR alpha-mediated transcriptional activation by human FOXP3. J. Immunol. Apr 2008; 180(7):4785–4792.

42. Zhang, F., G. Meng, and W. Strober. Interactions among the transcription factors Runx1, RORgammat and Foxp3 regulate the differentiation of interleukin 17-producing T cells. Nat. Immunol. Nov 2008;
43. Beutler BA. The role of tumor necrosis factor in health and disease. The Journal of Rheumatology. May 1999; 57:16–21

44. Liang Zhou, Jared E. Lopes, Mark M. W. Chong, Ivaylo I. Ivanov, Roy Min, Gabriel D. Victora, Yuelei Shen, Jianguang Du, Yuri P. Rubtsov, Alexander Y. Rudensky, Steven F. Ziegler & Dan R. Littman. TGF-beta-induced Foxp3 inhibits T(H)17 cell differentiation by antagonizing RORgammat function. Nature. May 2008; 453(7192):236–240

45. Kirsten J Flynn, Gabrielle Tbelz, John Daltman, Rafi Ahmed, David Lwoodland, Peter C Doherty. Virus-Specific CD8 + T Cells in Primary and Secondary Influenza Pneumonia. Immunity. June 1998; 8(6):683–691

46. U Muller, U Steinhoff, LF Reis, S Hemmi, J Pavlovic, RM Zinkernagel, M Aguet. Functional role of type I and type II interferons in antiviral defense. Science. Jun 1994; 264(5167):1918–1921

47. McElhaney JE, Xie D, Hager WD, Barry MB, Wang Y, Kleppinger A. T cell responses are better correlates of vaccine protection in the elderly. J Immunol. May 2006; 176(11):6333–6339.

Tables

Table 1

| Target or Query Sequence |
Table 2

| Organisms     | HMMER Hits | BLAST Hits |
|---------------|------------|------------|
| Homo sapiens  | 113        | 115        |
| Mus musculus  | 87         | 87         |
| **Total**     | **200**    | **202**    |

Table 3

Summary of the Gene Ontology Annotation
### Homo sapiens

| Gene Id          | Gene   | Protein                                    |
|------------------|--------|--------------------------------------------|
| ENSP00000365372.2| FOXP3  | Forkhead box P3                            |
| ENSP00000428952.1| FOXP3  | Forkhead box P3                            |
| ENSP00000365380.4| FOXP3  | forkhead box protein P3 isoform X3         |
| ENSP00000396415.3| FOXP3  | forkhead box protein P3 isoform X1         |
| ENSP00000365369.1| FOXP3  | forkhead box protein P3 isoform X1         |
| ENSP00000451208.1| FOXP3  | forkhead box protein P3 isoform X1         |

### Mus musculus

| Gene Id          | Gene   | Protein                                    |
|------------------|--------|--------------------------------------------|
| ENSMUSP00000041953.6| FOXP3  | forkhead box protein P3                    |
| ENSMUSP00000111403.1| FOXP3  | forkhead box protein P3                    |
| ENSMUSP00000111404.1| FOXP3  | forkhead box protein P3                    |
| ENSMUSP00000111405.1| FOXP3  | forkhead box protein P3                    |

**Figures**

![Image](image-url)

**Figure 1**

3D Structure of FOXP3 (Target or Query Sequence)
### Figure 2

Conserved Fork Head Domain (Multiple Sequence Alignment of FOXP3 in Two Organisms)
Figure 3

Sequence Motifs of FOXP3 (Motifs)
Figure 4

FOXP3 located at chromosome X in Human (Chromosome Location)

(a)

(b)

(c)

Figure 5

FOXP3 express in Human (Gene Expression)
Figure 6

FOXP3 interacts with different TF’s (Gene Network)