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The presentation of bacterial antigens to the CD4$^+$ T cells: The technique and advantage

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Abstract:
Antigen presentation is the process where a foreign body or a microbial antigen is presented to the T lymphocytes of the acquired immune system and eliminated from the body. For studying the mechanism of this process, antigen presentation assays can be applied. Three main stages of the mechanism should be studied: antigen uptake, antigen processing and antigen presentation. For these assays, the antigen, T lymphocytes, and antigen presenting cells are essential to carry out the assays. T cell hybridoma could be generated in the lab to be used instead of the primary T cells. This article focussed on a technical issue in immunology: the procedures and advantages. In this work, antigen presentation assays were highlighted by using two peptides from M5 protein of Streptococcus pyogenes as the antigen, T cell hybridomas specific for these peptides as T lymphocytes and macrophages as the antigen presenting cells. The results reflected the application of these assays and their advantages as a fundamental technique in immunology. That facilitates the study of immune responses against microbial antigens and designing vaccines against them.
Keywords: Antigen presentation assays; T cell hybridoma; Antigen uptake; Antigen processing, Antigen presentation.

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عرض الأنتجينات البكتيرية للخلايا التائية المساعدة : التقنية والأهمية

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الملخص:

عرض الأنتجين هو العملية التي يتم فيها تقديم جسم غريب أو مستضد ميكروبي إلى الخلايا الليمفاوية التائية للجهاز المناعي المكتسب والتخلص منه من الجسم. لدراسة آلية هذه العملية، يمكن تطبيق اختبارات عرض الأنتيجين. يجب دراسة ثلاث مراحل رئيسية من الآلية: إلتهام الأنتجين، هضم الأنتيجين وعرض الأنتجين. لإجراء هذه الإختبارات، يعتبر كل من الأنتجين والخلايا الليمفاوية التائية والخلايا العارضة للأنتجين أساس لإجراء هذه الإختبارات. يمكن تخليق خلايا تائية هجينة في المختبر لاستخدامها بدلًا من الخلايا التائية الأولية. ركزت هذه المقالة على تقنية مناعية من حيث التطبيق والأهمية. في هذا العمل، تم تسليط الضوء على اختبارات عرض الأنتجين باستخدام ببتيدين من بروتين للمكورات العقدية كأنتجين، وخلايا تائية هجينة خاصة بهذه الببتيدات كخلايا ليمفاوية تائية والبلعميات الكبيرة كخلايا عارضة للأنتجين. عكست النتائج تطبيق هذه الإختبارات وفوائدها كتقنية أساسية في علم المناعة، مما يسهل دراسة الإستجابات المناعية ضد الأنتجينات الميكروبية وتصميم اللقاحات ضدها.

الكلمات المفتاحية: اختبارات عرض الأنتجين؛ الخلايا الهجينة التائية؛ إلتهام الأنتجين؛ هضم الأنتجين؛ عرض الأنتجين

Introduction:

The mechanisms of antigen (Ag) processing and presentation is the main process of the acquired immune system. The main cells of the acquired immune system are T lymphocytes which consists of two types: helper T lymphocytes or $CD4^+$ T cells and cytotoxic T lymphocytes or $CD8^+$ T cells. The major molecules of the acquired immune system are the major histocompatibility (MHC) molecules, which consist of two classes: MHC class I and MHC class II. These molecules are loaded with the peptides after antigen processing, and the MHC/peptide complex are transported to the cell surface of antigen presenting cells (APCs) for recognition by the T

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lymphocytes. MHC class II molecules are loaded with antigenic peptides for presentation to the $CD4^+$ T cells and generating helper T cell responses. Whereas MHC class I molecules are loaded with antigenic peptides for presentation to the $CD8^+$ T cells and generating cytotoxic T cell responses (Robinson and Delvig, 2002; Vyas *et al.,* 2008; Pishesha *et al.,* 2022). The mechanism of Ag processing and presentation takes place within APCs. There are many immunological assays for measurement of immune responses against foreign and microbial antigens such as ELISA, ELISpot , FC , IF etc, but carrying out Ag presentation assays (Alvarez Freile *et al.,* 2023), *in vitro*, is a basic technique to study and understand the mechanisms of antigen uptake by professional APCs.

Within antigen uptake, professional APCs take up antigen via phagocytosis or pinocytosis or receptor-mediated endocytosis, by engulfing of a foreign antigen (for example, bacteria) from the extracellular fluid and that leads to the formation of phagosomes which deliver the ingested bacteria to a lysosome, where they are destroyed by degradative enzymes of the lysosome (Delvig *et al.,* 2002). Antigen processing is the process where the foreign or microbial antigen is degrades into small fragments (peptides) by the effects the proteases in APCs. The resulting peptides bind to MHC molecules which are transported to the surface of APCs surface for recognition by T cells (i.e., antigen presentation) via TCRs (Janeway *et al.,* 2005; Kotsias *et al.,* 2019).

Several proteolytic enzymes have been shown to participate in the processing of the antigens. They are in four main group families: aspartic proteases (e.g., cathepsins D and E), cysteine proteases (e.g., cathepsins L, S and H), serine and metallo-proteinases (Watts, 2001; Bryant *et al.,* 2002; Lennon-Dumenil *et al.,* 2002). After antigen uptake, proteins are subjected to disulphide reduction and unfolding , followed by degradation by proteinases at low pH and in endosomal compartments (Quin *et al.,* 2001; van Endert, 2019). Thus, antigen processing is an essential step for presentation. Antigen presentation is the delivering of antigenic peptides to the surface of APCs bound with MHC class I or MHC class II molecules for presentation to the T cells and inducing immune responses. Two main pathways of antigen presentation; MHC class I and class II pathways (Seliger *et al.,* 2000). Within the MHC class II antigen presentation pathway, there is a conventional pathway, known as a classical pathway, where the nascent (newly synthesized) MHC class II molecules are used. There is another type, known as a

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recycling pathway which occurs by internalization of the pre-existing cell surface MHC class II/peptide complexes and recycling through endosomes, where peptides are exchanged and recycled for presentation at the cell surface (Robinson and Delvig, 2002; Vyas *et al.*, 2008; Hilbert and Roche, 2002). This article aims to highlight the principles of antigen presentation assays to demonstrate the procedure of these assays for studying presentation of the bacterial antigen and immune responses against them. Applying these assays lead to deep understating of the mechanisms of immune responses against microbes and highlight the technical issues in immunology and microbiology.

Materials and Methods:

Antigens

$M5_{1-35}$ and $M5_{359-388}$ synthetic peptides from M5 protein of Streptococcus pyogenes were used as T cell epitopes. *Streptococcus pyogenes* (The Manfredo strain of type 5) was used as a source of an antigen.

Mouse immunization

Four C57BL/6 mice were immunized in their footpad with the $M5_{1-35}$ and $M5_{359-388}$ synthetic peptides (both at 6 mg/ml and two mice for each peptide) of streptococcal M5 protein. 150 µg/ml of each peptide was mixed with 1:1 in Titremax adjuvant and injected to the mouse.

Tissue culture

RF10 medium was used for growing the T cells, Bone marrow (BM) medium for macrophages growth and for growing the bacteria. RF10 medium, Bone marrow (BM) medium and microbiological culture medium were prepared as detailed in (Alhoderi, 2023). T cell lines and T cell hybridomas specific for each peptide were generated as indicated in (Alhoderi, 2023). Bone marrow-derived macrophages were grown in Petri dishes with the culture media specific for them and activated by treatment with 1 ng/ml interferon-γ and incubated overnight at 37 °C.

Antigen presentation assays

Ag uptake assay

This part will examine how the antigen is uptake by APC. Three systems were used to study the antigen uptake of $M5_{1-35}$ peptide by BM. This involves pre-fixation where the cells will be fixed before adding the antigen. Pre-fixation looks at the structure of the antigen and how APC can uptake it. Antigen uptake also involves post-

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fixation where the cell will be fixed after adding the antigen. This will study the kinetics of antigen presentation (Canaday *et al.,* 2003; Musson *et al.,* 2003).

In pre-fixing of APCs, macrophages were grown in a flat-bottom 96-well microtiter plates for 1 h at 37 °C in the incubator to adhere and then fixed before adding antigen. Three doses of peptide at 2mg/ml (0.8, 4 & 20µg/ml) and three doses of viable or HK *S. pyogenes* were used. Whereas, in post-fixation system, macrophages were fixed after adding the antigen. Un-fixed antigen-pulsed macrophages and/or fixed non-antigen-pulsed macrophages were used as controls. The cells were incubated for 5 hrs after adding antigen then fixed in 1% paraformaldehyde for 5 mins to block Ag uptake by BMM. Fixation was stopped with 0.05% Gly-Gly and the cells washed three times with HANKS (balanced salt solution). Then T-cell hybridomas (4×10^4/well) were added to the antigen-pulsed macrophages, and the plate was incubated for 24 hours. The viability of the cells was confirmed by light microscopy before putting them in -30 freezer. After 24 hours of freezing, culture supernatants were thawed and used in CTLL assay.

Antigen processing assay

This part will focus on the role of proteases in processing of the antigen. The protease families which are enrolled in antigen processing are: Cysteine and Aspartic which can work for minor processing. Serine and Metallo that are required for the major processing [8]. This assay aims to show the effect of proteinase inhibitors on presentation of the antigen and to determine which families of enzymes are involved in processing of the immunogenic peptide from the antigen and the effect of treatment of macrophages with different proteinase inhibitors covering the enzymatic families. The inhibitors of these families are used to determine whether lysosomal enzymes are required for presentation of the antigenic epitopes. In this work, pepstein used as an example for the assay procedure. Pepstatin is used as an inhibitor of aspartic proteinases (Bond, 2019). In this assay, macrophages were grown in a flat-bottom 96-well microtiter plates for 1 h at 37 °C in the incubator to adhere and then treated with a proteinase inhibitor of each proteinase family for 30 mins. After the incubation time with the inhibitor, antigen and peptides were added in a triplicate of three doses of M5 peptide and three doses of HK-S and incubated for up to 5 hrs. Then T-cell hybridomas (4×10^4/well) were added to the experimental plate, and the plate was incubated for 24 hrs at 37 °C

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in the incubator. The viability of the cells was seen under the microscope. Then, the plate was put in -30 freezer. After 24 hrs of freezing, culture supernatants were thawed and used in CTLL assay. Cells without inhibitor were used as a negative control and SEB was used as positive control for this assay.

Antigen presentation assay

BMMs at $4*10^4$ per well were cultured in a flat-bottom 96-well microtiter plates and incubated in the incubator (37 °C) for 1 h to allow them to adhere. Two metabolic inhibitors were used for determining antigen presentation pathways; brefeldin A which affects the Golgi network, and cycloheximide which inhibits protein synthesis. Both inhibitors block the classical antigen presentation pathway by preventing accumulation of newly synthesized MHC-II molecules. Brefeldin A was at 1mg/ml and cycloheximide was 40mM. Macrophages were pre-treated with the inhibitors and incubated for 30 min. The metabolic inhibitors were removed by washing with HANKs three times, followed by fixing the cells with 1% paraformaldehyde for 5 min. Fixation was stopped with 0.05% Gly-Gly and the cells were washed three times. The viability of macrophages should be confirmed by light microscopy to confirm that the inhibitors are safe for the cells. Then the antigen was added, and a 5 hrs further incubation was allowed. After that, the cells were fixed as above, TCHs at $4*10^4$ per well were added and incubated for 24 h in the incubator. The plate was frozen at -30 °C overnight. Culture supernatants were thawed and collected to measure responses of TCHs using CTLL assay as explaining below. SEB was used as a positive control for this assay.

CTLL assay

The response of TCHs was measured for each Ag presentation assay and CTLL assay was applied. CTLL assay was done by using of CTLL-2 cells, which are cytotoxic T cells line derived from mouse and are dependent on IL-2 for their growth. Therefore, they are used to assay for IL-2 secretion by TCHs. The assay is carried out by washing CTLL-2 cells three times and cultured them in a flat-bottomed 96-well plate (in triplicate wells at 4×10^4/well) overnight without IL-2. The supernatants of the culture of Ag presentation assays were added to the plate wells. The response of TCHs in this assay is measured as the amount of IL-2 secreted as a proliferation of CTLL-2 cells. A triplicate well with IL-2 were used as a positive control and wells without IL-2 as a negative control. Positive results

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are considered if the amount of IL-2 in the experimental wells is more than two-fold of the negative control.

Results:

Ag presentation assays including Ag uptake, processing and presentation were carried out to demonstrate the basic technique for studying the mechanisms of Ag processing and presentation using a bacterial antigen ($M5_{1-35}$ and $M5_{359-388}$ synthetic peptides) from an M5 protein of Streptococcus pyogenes and a viable or heat-killed bacteria as a source of M5 protein. T cell hybridomas specific for each peptide were used as helper T cells (CD4+ T cells) and BMMs from the immunized mice were used as APCs. The key results presented here is for demonstration the procedure of the technique, its aim and significance.

Antigen uptake assay

T cell hybridoma specific for the peptide was used to test the uptake of peptide and the viable streptococci as a source of M5 protein via pre-fixation, post-fixation and non-fixation of macrophages to look at antigen uptake. The result of TCH-9 is shown here representing unresponsiveness when using pre- fixed macrophages in comparison with non- and post-fixed macrophages (Figure 1).

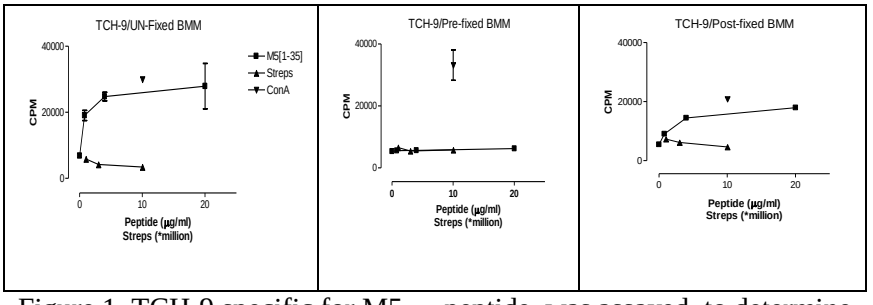

Figure 1. TCH-9 specific for $M5_{1-35}$ peptide was assayed to determine the uptake of $M5_{1-35}$ peptide and the viable streptococci as a source of M5 protein. Pre-fixed BMM (middle) showed that no uptake of the peptide. Whereas in post-fixed BMM (right), Ag uptake was seen, and no response was reported with the bacteria. Non-fixed macrophages were used as a control for the uptake system (left) and ConA was used for assay control.

Antigen processing assay

As mentioned in the methods, Ag processing assay is done to demonstrate the effect of proteinase inhibitors on presentation of the antigen and to determine which enzymes are engaged in processing

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of the antigen. In this work as a technical subject, pepstein used as an example of the assay procedure. The results of this assay showed that no effect of pepstein on the processing of M5 synthetic peptides and the bacteria (date not shown).

Antigen presentation pathways

The metabolic inhibitors (brefeldin A and cycloheximide) were used to investigate the antigen presentation pathways of $M5_{1-35}$ peptide. TCH-9, IFNγ-treated BMM, and HK bacteria were used. The result showed a significant response of the peptide with both inhibitors and no response to the HK streptococci (Figure 2; right and left, respectively). CTLL control was done for Ag uptake, processing and presentation assays and presented a low background (Figure 2; middle). A CTLL control assay was done for each assay.

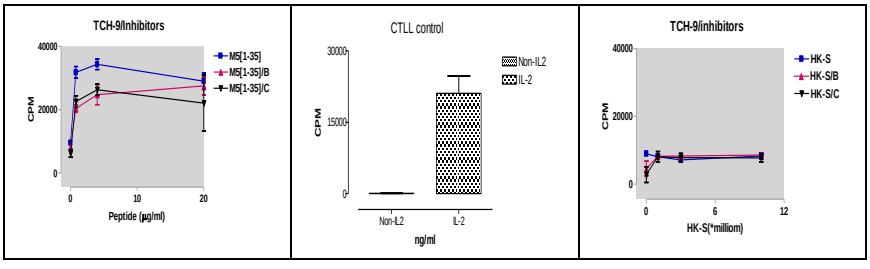

Figure 2. Brefeldin A (B) and cycloheximide (C) were used to investigate the antigen presentation pathways of $M5_{1-35}$ peptide using TCH specific for the peptide. The procedure mentioned in the methods for this assay was applied. The data showed TCH response to the peptide with both inhibitors (left). This result might indicate that $M5_{1-35}$ peptide is presented by the recycling MHC-II molecules. No response to the HK streptococci was seen (right panel). A low background (Non-IL2) was shown by CTLL assay (middle).

Discussion:

The presented work aimed to demonstrate the technical issues related for applying Ag uptake, Ag processing and Ag presentation to study the mechanisms of presentation of bacterial antigen, as understanding the mechanisms of antigen processing and presentation is essential to realise what are the factors that influence the effective processing and presentation of microbial antigens such

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as M5 protein of *S. pyogenes* (von Delvig *et al.*, 2003a), Salmonella invasion protein (SipC) of *Salmonella typhimurium* (Musson *et al.*, 2002) Protective antigen of *Bacillus anthracis* (Musson et al., 2003), and V antigen of *Yersinia pestis* (Shim *et al.*, 2006). That leads to new contributions to design of bacterial vaccines against infectious diseases (Kotloff *et al.*, 2004; Musson *et al.*, 2014).

The unresponsiveness of TCHs to recognize the bacteria (viable and HK) in the presented assays may attribute to several factors. One of the most important that the mice were not immunized with M5 protein of Streptococcus pyogenes as they were immunized with the synthetic M5 peptides instead. Therefore, generation of T cell hybridomas specific for the streptococcal M5 protein under study is the fundamental step to study Ag processing and presentation to T cells, and that what have been applied in previous such studies with different antigens and different bacteria (Musson *et al.*, 2002; von Delvig *et al.*, 2003a; Musson *et al.*, 2003; Shim *et al.*, 2006; Mimure *et al.*, 2007; Musson *et al.*, 2014). The procedures of Ag presentation assays presented in this paper have been developed and applied in several previous studies (Musson *et al.*, 2002; von Delvig *et al.*, 2003a; Musson *et al.*, 2003; Shim *et al.*, 2006; von Delvig *et al.*, 2003b; von Delvig *et al.*, 2005; Ascough *et al.*, 2014; Ingram *et al.*, 2015; Reynolds *et al.*, 2015; Ascough *et al.*, 2016) The results of the current study showed that the T cell hybridomas specific for both M5 synthetic peptides of *Streptococcus pyogenes* did recognise both immunogenic peptide from this protein and did not recognize HK-S and viable bacteria. That means a TCH specific for the bacterial M5 protein should be generated instead, and that needs immunization of mice with M5 protein of the bacteria to generate M5 specific T cell hybridomas. By using T cell hybridomas specific of the antigen we can study the mechanisms of Ag processing and presentation to T cells (Musson *et al.*, 2002; Musson *et al.*, 2003; Shim *et al.*, 2006). This work demonstrates the importance of generating T cell hybridoma as a continues growing cells for studying the T cells immune responses against the bacterial antigens. Ag presentation assays can be applied in several topics in terms of study the effect of different factors on the presentation of the antigens including bacteria, viruses, parasites. DCs or B cells can be used also as professional APCs. These assays can be also demonstrated by imaging which facilitates seeing the stages of each process (Lau and Elliott, 2025)

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Conclusion:

Applying antigen presentation assays for study the mechanism of Ag processing and presentation allows the researchers for deep understanding of the mechanism. In the current work, antigen presentation assays were highlighted by using bacterial antigen, T cell hybridomas specific for this antigen as T lymphocytes and macrophages as the antigen presenting cells. The results demonstrated the application of these assays and their advantages as a fundamental technique in immunology. These assays help the researchers to study of immune responses against microbial antigens and designing vaccines against them. In conclusion, these assays are a cellular (cell culture) technique, and fundamental in immunology.

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References:

Alhoderi, J. (2023). Generation of T cell hybridoma as a technique for study the immune response against bacterial infections. *Journal of Pure & Applied Sciences, 22,* 185–190.

Álvarez Freile, J., Qi, Y., Jacob, L., Lobo, M. F., Lourens, H. J., Huls, G., & Bremer, E. (2023). A luminescence-based method to assess antigen presentation and antigen-specific T cell responses for in vitro screening of immunomodulatory checkpoints and therapeutics. *Frontiers in Immunology, 14,* Article 1233113.

Ascough, S., Ingram, R.J., Chu, K.K., et al., (2014). Anthrax lethal factor as an immune target in humans and transgenic mice and the impact of HLA polymorphism on CD4+ T cell immunity. *PLOS Pathogens,10, e1004085*.

Ascough, S., Ingram, R.J., Chu, K.K.Y., et al., (2016). CD4+T Cells Targeting Dominant and Cryptic Epitopes from Bacillus anthracis Lethal Factor. *Frontiers in Microbiology, 6,* 1506.

Bond, J.S. (2019). Proteases: History, discovery, and roles in health and disease. *J Biol Chem., 294,*1643-1651.

Bryant, P.W., Lennon-Dumenil, A-M., Fiebiger, E., Lagaudriere-Gesbert, C., & Ploegh, H. (2002). Proteolysis antigen presentation by MHC class II molecules. *Advances in Immunology, 80,* 70-114.

http://www.doi.org/10.62341/jaha1707

Canaday, D.H., Gehring, A.J., Leonard, E.G., Eilertson, B., Schreiber, J.R., Harding, C.V., & Boom, W.H. (2003). T-cell hybridomas from HLA-transgenic mice as tools for analysis of human antigen processing. *Journal of immunological methods*, *281*, 129-142.

Delvig, A.A ., Bailey, E ., Gibbs, D.M., & Robinson, J.H. (2002). The route of bacterial uptake by macrophages influences the repertoire of epitopes presented to CD4 T cells. *Eur. J. Immunol.*, *32*, 3714–3719.

Hiltbold, E.M., & Roche, P.A. (2002).Trafficking of MHC class II molecules in the late secretory pathway. *Current Opinion in Immunology*, *14*, 30-35.

Ingram, R.J., Ascough, S., Reynolds, C.J., et al., (2015). Natural cutaneous anthrax infection, but not vaccination, induces a CD4(+) T cell response involving diverse cytokines. *Cell & Bioscience*, *5*, 20.

Janeway, C.A ., Travers, P., Walport, M., & Shlomchik, M.J . Immunobiology. (2005). 6th Ed. Garland Science Publishing.

Kotloff, K.L ., Corretti, M ., Palmer, K ., Campbell, J.D., Reddis, M.A., Hu, M.C., Wasserman, S.S., & Dale, J.B. (2004). Safety and Immunogenicity of a Recombinant Multivalent Group A Streptococcal Vaccine in Healthy Adults: Phase 1 Trial . *JAMA*, *292*, 709-715.

Kotsias, F., Cebrian, I., & Alloatti, A. (2019). Antigen processing and presentation. In International Review of Cell and Molecular Biology, 348, Pages 69-121, Academic Press. ScienceDirect.

Lau, D., Elliott, T. (2025). Imaging antigen processing and presentation in cancer. *Immunother Adv., 5,* ltaf002.

Lennon-Duménil, A.M., Bakker, A.H., Wolf-Bryant, P., Ploegh, H.L., & Lagaudrière-Gesbert, C. (2002). A closer look at proteolysis and MHC-class-II-restricted antigen presentation. *Current opinion in immunology*, *14*, 15-21.

Mimure, Y., Mimure-Kimure, Y., Doores, K., Golgher, D., Davis, B.G., Dwek, R.A., Rudd, P.M. & Elliott, T. (2007). Folding of an MHC class II-restricted tumor antigen controls its antigenicity via MHC-guided processing. *PNAS*, *104*, 5983-5988.

Musson, J.A., Hayward, R.D., Delvig, A.A., Hernan, R., Hormaeche, C.E., Koronakis, V., & Robinson, J.H. (2002). Processing of viable Salmonella typhimurium for presentation

http://www.doi.org/10.62341/jaha1707

of a CD4 T cell epitope from Salmonella invasion protein (SipC). *Eur. J. Immunol., 32,* 2664-2671.

Musson, J. A., Reynolds, C. J., Rinchai, D., Nithichanon, A., Khaenam, P., Favry, E., … Robinson, J. H. (2014). CD4+ T Cell Epitopes of FliC Conserved between Strains of Burkholderia : Implications for Vaccines against Melioidosis and Cepacia Complex in Cystic Fibrosis. *The Journal of Immunology*, *193*(12), 6041–6049.

Musson, J.A ., Walker, N ., Flick-Smith, H ., Williamson, D., & Robinson JH (2003). Differential processing of CD4 T- cells epitopes from the protective antigen of Bacillus anthracis. *J. Bio. Chem., 278,* 52425-52431.

Pishesha, N., Harmand, T.J. & Ploegh, H.L. (2022). A guide to antigen processing and presentation. *Nat Rev Immunol., 22,* 751–764.

Quin, S.J ., Seixas, E.M ., Cross, C.A ., Berg, M ., Lindo, V ., Stockinger, B., & Langhorne, J. (2001). Low CD4+ T cell responses to the C-terminal region of the malaria merozoite surface protein-1 may be attributed to processing within distinct MHC class II pathways. *Eur J Immunol., 31,* 72-81.

Reynolds, R., Goudet, A., Jenjaroen, K., et al., (2015). T Cell Immunity to the Alkyl Hydroperoxide Reductase of Burkholderia pseudomallei: A Correlate of Disease Outcome in Acute Melioidosis. *Journal of Immunology, 194*, 10.

Robinson, J.H & Delvig, A.A. (2002). Diversity of MHC antigen presentation. *Immunology, 105,* 252-262.

Seliger, B., Maeurer, M.J., & Ferrone, S (2000). Antigen-processing machinery breakdown and tumor growth. *Immunology Today, 21,* 457-464.

Shim, H-K., Musson, J.A., Harper, H.M., McNeill, H.V., Walker, N., Flick-Smith, H., von Delwig, AA., Williamson, E.D., & Robinson, J.H. (2006). Mechanisms of major histocompatibility complex class II-restricted processing and presentation of the V antigen of Yersinia pestis. *Immunology, 119,* 385–392.

van Endert, P., editor. Antigen Processing : Methods and Protocols. (2019) . 2nd Ed. Springer New York.

von Delvig, A.A ., Ramachanda, L ., Harding, C.V., & Robinson, J.H. (2003a). Localization of peptide/complexes in macrophages following antigen processing of viable Streptococcus pyogenes. *Eur. J. Immunol., 33,* 2353–2360.

http://www.doi.org/10.62341/jaha1707

von Delvig, A ., Musson, J.A ., McKie, N ., Gray, J., Robinson, J.H. (2003b). Regulation of peptide presentation by major histocompataibility complex class II molecules at the surface of marophages. *Eur J Immunology 33*, 3359-3366.

von Delvig, A ., Musson, J.A ., Gray, J ., McKie, N & Robinson, J.H. (2005). Major histocompataibility complex class II molecules prevent destructive processing of exgonous peptides at the surface of marophages for presentation to CD4. *Immunology 114* , 194-203.

Vyas, J.M., Van der Veen, A.G., & Ploegh, H.L. (2008). The known unknowns of antigen processing and presentation. *Nature reviews. Immunology, 8,* 607-618.

Watts, C. (2001). Antigen processing in the endocytic compartment. *Curr Opin Immunol., 13,* 26-31.