Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution: Lankenau Institute for Medical Research**

2. **Reporting Period (start and end date of grant award period): 1/1/2010 – 12/31/10**

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees): Tam Mai-Nguyen**

4. **Grant Contact Person's Telephone Number: 484-476-2755**

5. **Grant SAP Number: 4100050898**

6. **Project Number and Title of Research Project: Project 2, The Role of IDO in B Cell Activation and Memory**

7. **Start and End Date of Research Project: 1/1/2010 to 12/31/2010**

8. **Name of Principal Investigator for the Research Project: Laura Mandik-Nayak, PhD**

9. **Research Project Expenses**.

   9(A) Please provide the amount of health research grant funds spent on this project for the entire duration of the grant, including any interest earned that was spent:

   $ 120,764.82 (DC: $73,239.82; IC: $47,525)

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project	Cost
Mandik-Nayak	P.I.	20%	$22,200.02

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project
Pigott	Biomedical Research Assistant	25
Galanti	Student intern	100

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No____X_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes____X_____ No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
IDO, A New Target in the Pathogenesis and Treatment of Mitral Valve Disease	✓NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)	Nov. 2010	$ 453,750	$
IDO, A New Target in the Pathogenesis and Treatment of Mitral Valve Disease	☐NIH ☐ Other federal (specify:_____ _____) ✓ Nonfederal source (specify: Alliance for Lupus Research)	Aug. 2010	$ 399,706	not funded
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes___X_____ No_____

If yes, please describe your plans:

We have recently received a NIH RO1 grant to follow up on our finding that IDO plays a role in B cell-mediated immune responses. This grant focuses on the role of IDO in driving autoantibody-mediated joint inflammation in a mouse model of Rheumatoid arthritis. We have also applied for additional funding from the NIH in the form of an R21 application to extend these findings to a serious co-morbidity in Rheumatoid arthritis, mitral valve disease. If this grant is not funded, we will continue to seek additional funding in the future to support this line of research.

12. Future of Research Project. What are the future plans for this research project?

The work supported by this grant focused on the role of IDO1 in B cell activation and memory in an *in vivo* model antigen system. Our data demonstrated that IDO1 plays a role in the generation of a high affinity isotype-switched memory B cell response. Furthermore, these studies showed that IDO1 is dispensable when B cells receive a secondary stimulus through TLRs. This suggests a potential pathway by which IDO acts to support B cell-mediated immunity. Future studies in the lab will include determining the mechanism by which IDO1 acts to influence B cell activation and memory, as well as investigating the role of a related immunomodulatory enzyme, IDO2, both in *in vitro* and *in vivo* model systems.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes__X_____ No_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male				
Female	1			
Unknown				
Total	**1**			

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic	1			
Unknown				
Total	**1**			

	Undergraduate	Masters	Pre-doc	Post-doc
White	1			
Black				
Asian				
Other				
Unknown				
Total	**1**			

14. **Recruitment of Out-of–State Researchers**. Did you bring researchers into Pennsylvania to carry out this research project?

    Yes_____ No____X_____

    If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. **Impact on Research Capacity and Quality**. Did the health research project enhance the quality and/or capacity of research at your institution?

    Yes___X_____ No_____

    If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

    The data generated from this research project has led to new collaborations with investigators at my institution. We are also in the process of writing a review article, incorporating some of the generated data.

16. **Collaboration, business and community involvement.**

    16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

    Yes_____ No____X_____

    If yes, please describe the collaborations:

    16(B) Did the research project result in commercial development of any research products?

    Yes_____ No_____X_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No_____X_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant application's strategic plan). Summarize the progress made in achieving these goals, objectives and aims for the entire grant award period. Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

Rheumatoid arthritis (RA) is a chronic and debilitating inflammatory autoimmune disease of unknown etiology. As with other autoimmune disorders, elevated tryptophan catabolism has been detected in RA patients. This is indicative of activation of the immunomodulatory enzyme indoleamine 2,3-dioxygenase (IDO); however, the role that IDO plays in the disease process is not well understood. The current paradigm for IDO function is immunosuppressive, providing an important inhibitory role to limit effector T cell responses. In contrast, the role of IDO in B cell-mediated responses has not been addressed. The widely held belief that IDO acts solely in a T cell immunosuppressive role has led to the general assumption that inhibition of IDO activity should exacerbate autoimmune disorders. However, using the K/BxN mouse model of RA and 1-methyl-tryptophan (1MT), an inhibitor of IDO, we found that inhibiting IDO activity had the unexpected consequence of ameliorating, rather than exacerbating arthritis symptoms. This alleviation of joint inflammation was not due to an altered T cell response, but rather resulted from a diminished autoreactive B cell response. We have preliminary data demonstrating that 1MT is also able to inhibit B cell responses to immunization with model antigens, suggesting for the first time an important role for IDO in directing B cell responses to both self and foreign antigens. *Our working hypothesis is that IDO is not simply an immunosuppressive enzyme, but rather plays a more complex role in modulating inflammatory responses by directing the immune profile of B cell responses.*

Specific Aims:

Aim 1. Determine the role of IDO in the development of B cell memory.

Aim 2. Define the mechanism by which IDO drives B cell activation in response to foreign antigens.

Aim 1. Determine the role of IDO in the development of B cell memory.

Loss of IDO activity inhibits magnitude of secondary immune response

The memory B cell response is characterized by a rapid appearance of antigen-specific antibody in response to a re-challenge with antigen. While the phenomenon of B cell memory has been extensively studied, the factors mediating its set-up and maintenance remain poorly defined. Our preliminary data showed that IDO influences the development of a primary antibody response to both self and model antigens, suggesting that IDO may also play a role in the development of memory B cell responses. To determine the role of IDO in memory B cell responses *in vivo*, we compared the B cell recall response to immunization in C57BL/6 mice in the presence and absence of IDO activity. Two different methods were used to inhibit IDO activity. First, the small molecule inhibitor that targets IDO, D/L-1MT, was used to pharmacologically block IDO activity. Second, mice genetically deficient in IDO ($IDO1^{-/-}$ mice) were used.

$IDO^{-/-}$ and carrier and 1MT-treated C57BL/6 mice were immunized i.p. with the T cell-dependent antigen (4-hydroxy-3-nitrophenyl) acetyl (NP) – conjugated to keyhole limpet hemocyanin (KLH) precipitated in alum. Eight weeks later, the mice were re-challenged i.p. with NP-KLH. Serum was collected from the immunized mice once a week for 4 wk following the primary response and once a week for 4 wk following the secondary response to determine

the amount and isotype of high vs. low affinity anti-NP antibody present. Carrier-treated C57BL/6 mice generated a good secondary response to NP-KLH, showing a 3-fold increase in total anti-NP titers (Fig. 1a). In contrast, C57BL/6 mice treated with 1MT and IDO1$^{-/-}$ mice did not show an increase in total anti-NP titers (Fig. 1a). Although the primary response is not significantly different between carrier and 1MT-treated C57BL/6 or IDO$^{-/-}$ mice at this early timepoint following immunization, the secondary response generated in carrier-treated C57BL/6 mice is significantly higher than the secondary response generated in 1MT-treated C57BL/6 mice or IDO$^{-/-}$ mice (Fig. 1a). Together, these data suggest that IDO plays a role in generating a secondary immune response.

At the termination of the experiment, 30 days after the second immunization, spleens from the immunized mice were harvested, and frozen sections examined for the presence of antibody secreting cell (ASC) foci and germinal centers. NP-specific B cells were identified by staining for Igλ-expression and germinal centers by staining with PNA (Fig. 2). By this time in the response few ASC foci are found in the spleens of carrier-treated C57BL/6 mice. Similar low numbers of ASC foci were also detected in 1MT-treated C57BL/6 and IDO$^{-/-}$ mice (Fig. 2). Small, loosely aggregated germinal centers were detected in carrier-treated C57BL/6 mice 30 days post-secondary injection. In contrast, well-defined PNA^{+} germinal center clusters were present in almost every follicle in the 1MT-treated C57BL/6 and IDO$^{-/-}$ mice (Fig. 2). This suggests that IDO may play a role in dissolving the germinal center response at the end of the immune response.

Mice lacking IDO activity are able to generate high affinity Ig during secondary immune response

One of the hallmarks of a secondary immune response is the rapid generation of high affinity antibodies. To determine the relative affinity of the anti-NP antibodies generated in IDO$^{-/-}$ and carrier and 1MT-treated C57BL/6 mice, their binding to differently haptenized carrier proteins (NP_{16}-BSA vs. NP_3-BSA) was compared (Fig. 1b). The NP_{16}-BSA ELISA detects low and high affinity antibodies while the NP_3-BSA ELISA detects only high affinity antibodies. Carrier-treated C57BL/6 mice generated a robust high affinity anti-NP response to secondary immunization (Fig. 1b). High affinity anti-NP antibodies were also present in IDO$^{-/-}$ and 1MT-treated C57BL/6 mice, although at a lower magnitude than found in carrier-treated mice (Fig. 1b). This indicates, that while IDO plays a role in generating a robust secondary immune response, it does not inhibit the process of affinity maturation.

Loss of IDO activity affects isotype-switching during secondary immune response

An important component of effective secondary immune responses is the generation of high titers of isotype-switched serum antibody. The response to NP is predominantly associated with switching to IgG_1, although other IgG's are also present. To determine if IDO is necessary for isotype-switching, the isotype of anti-NP Ig generated in response to a primary and secondary immunization with NP-KLH was compared in carrier and 1MT-treated C57BL/6 and IDO$^{-/-}$ mice (Fig. 3). In agreement with the literature, the majority of anti-NP serum Ig in carrier-treated C57BL/6 mice was IgG_1. Titers of IgM, $IgG_{2a/c}$, IgG_{2b}, and IgG_3 were also present. However, only IgG_1 and $IgG_{2a/c}$ showed a significant increase in the secondary response (Fig. 3). Titers of

anti-NP IgM, IgG_1, $IgG_{2a/c}$, IgG_{2b}, and IgG_3 were also detected in the serum of 1MT-treated C57BL/6 and $IDO^{-/-}$ mice, with IgG_1 again showing the highest levels. In contrast to carrier-treated mice, 1MT-treated C57BL/6 and $IDO^{-/-}$ mice did not show an increase in anti-NP IgG_1 titers upon secondary immunization (Fig. 3). Titers of anti-NP $IgG_{2a/c}$ did increase between the primary and secondary response in 1MT-treated C57BL/6 mice, but not $IDO^{-/-}$ mice. In both cases; however, the titers remained lower than those generated in carrier-treated mice. No differences were detected between the different groups for anti-NP IgM, IgG_{2b}, or IgG_3 (Fig. 3). These data demonstrate that IDO plays a role in generating anti-NP IgG_1 and $IgG_{2a/c}$ responses upon secondary immunization.

<u>Aim 2. Define the mechanism by which IDO drives B cell activation in response to foreign antigens.</u>

Loss of IDO activity inhibits early B cell responses to NP-KLH in Titermax

In preliminary experiments, immunization of mice with NP-KLH in alum demonstrated a deficiency in anti-NP antibody production in IDO1 deficient mice. This suggested that IDO1 is necessary for the generation of a productive B cell response. However, it was not clear from these studies what role IDO1 was playing. Experimentally, adjuvants have been used to define critical components of pathways required for T and B cell immune responses by overcoming activation defects in knockout mouse strains. To begin to define the conditions under which IDO1 deficiency affects B cell responses, we used mice genetically deficient in IDO1 together with three different adjuvant systems.

To start, we used the titermax adjuvant system, a particulate adjuvant that is similar to the adjuvant alum used in our preliminary experiments. Titermax, like alum, induces chemokine production by macrophages and monocytes, leading to cell recruitment and indirect activation of dendritic cells (DCs). These experiments first allowed us to test whether our preliminary result showing reduced antibody production in IDO1 deficient mice in response to immunization with NP-KLH in alum is also found with a different, but similar adjuvant. Second, it gave us insight into the pathway that requires IDO1 for serum Ig responses.

C57BL/6 mice genetically deficient for IDO1 (IDO1 ko) and C57BL/6 control mice were immunized i.p. with 100μg NP-KLH emulsified in titermax (Fig. 4). Serum was collected at days 0, 6, 15, 22, and 29 and tested for the presence of anti-NP antibodies by ELISA. Anti-NP IgM titers were elevated by day 5 and remained steady for several weeks in both control and IDO ko C57BL/6 mice. In C57BL/6 control mice, titers of anti-NP IgG_1, $IgG2_{a/c}$, IgG_{2b}, and IgG_3 were also high starting on day 5 and then continued to increase over the next few weeks. In contrast, in IDO ko C57BL/6 mice, reduced levels of anti-NP IgG_1 and IgG_{2b} and undetectable $IgG_{2a/c}$ and IgG_3 were found at day 5. IDO ko mice did generate anti-NP IgG, as titers reached comparable levels to control C57BL/6 mice by day 10 for IgG_1 and IgG_{2b} and day 15 for $IgG_{2a/c}$ and IgG_3 (Fig. 4). These data suggest that IDO may play a role early in the response, but that additional factors are able to compensate for its loss later in the response.

Loss of IDO activity does not inhibit B cell responses to NP-KLH in CFA

To determine if an adjuvant that stimulates Toll-like receptors can overcome the defect in IDO ko mice, the model antigen NP-KLH was used in combination with complete Freund's adjuvant (CFA). CFA, derived from the microbacterium *m. tuberculosis*, stimulates Toll-like receptors (TLRs) to directly activate DCs and B cells, allowing us to test whether loss of IDO activity affects B cell activation directly.

IDO1 ko and C57BL/6 control mice were immunized i.p. with 100μg NP-KLH emulsified in CFA (Fig. 5). Serum was collected at days 0, 6, 15, 22, and 29 and tested for the presence of anti-NP antibodies by ELISA. The vast majority of anti-NP Ig in both control and IDO1 ko C57BL/6 mice was of the IgG_1 isotype. Titers of anti-NP IgG_1 were present at day 5 and then increased throughout the remainder of the experiment. Anti-NP $IgG2_{a/c}$, IgG_{2b}, and IgG_3 were also detectable at day 5, increased up to day 10-15, and then plateaued. Titers of anti-NP IgM were first detected at day 5 and peaked by day 10. No differences were detected between C57BL/6 mice with and without IDO1 (Fig. 5). Therefore, these data demonstrate that stimulation through TLRs is able to bypass the defect in IDO1 ko mice. This suggests that the defect in IDO1 ko mice is in the initial induction of serum antibody responses and can be overcome by TLR stimulation.

Loss of IDO activity does not inhibit B cell responses to NP-CGG in Alum

Due to the differences seen with NP-KLH in combination with the adjuvants alum and titermax compared to CFA, we used a second NP conjugate, NP-chicken gamma globulin (CGG). NP-CGG, like NP-KLH, is a T-dependent antigen. B cells will still respond to the hapten NP; however T cell help will be generated in response to CGG, as opposed to KLH. In the future, these different hapten/carrier systems could be used to distinguish the role of IDO in T vs. B cells during primary and memory immune responses.

IDO1 ko and C57BL/6 control mice were immunized i.p. with 100μg NP-CGG in alum (Fig. 6). Serum was collected at days 0, 6, 15, 22, and 29 and tested for the presence of anti-NP antibodies by ELISA. Serum anti-NP IgM, IgG_1, $IgG_{2a/c}$, and IgG_{2b} responses were first detected at day 5, peaked at day 10, and then plateaued. Very little IgG_3 was present and didn't appear until day 10. Overall, titers of anti-NP were lower in response to NP-CGG in alum (Fig. 6) than they were to NP-KLH, in either alum (as shown in preliminary data) or titermax (Fig. 4). Surprisingly, and in contrast to immunization with NP-KLH in alum or titermax, no difference was seen between IDO1 ko and control C57BL/6 mice (Fig. 6). It is unclear whether the lack of difference is due to the difference in carrier used (KLH compared to CGG) or the fact that the overall antibody response to NP-CGG was lower than to NP-KLH.

Conclusions

The memory B cell response is characterized by a rapid appearance of antigen-specific antibody in response to a re-challenge with antigen. While the phenomenon of B cell memory has been extensively studied, the factors mediating its set-up and maintenance remain poorly defined. The experiments described in the first aim of this project describe a novel role for IDO in the generation of B cell memory. Comparing the anti-NP B cell response in mice with and without IDO activity demonstrated that IDO helps shape the high affinity isotype-switched Ig repertoire

characteristic of a productive memory B cell response. Mice lacking IDO activity, either genetically or through pharmacologic inhibition, exhibited reduced titers of NP-specific serum antibody following re-challenge with antigen. IDO was not required for the development of high affinity isotype-switched antibody; however it was required for "boost" effect following secondary immunization.

As a group, adjuvants are thought to work by stimulating innate immune receptors on dendritic cells and monocytes, stimulating the production of chemokines and cytokines necessary for productive adaptive immune response. Just as different antigens can trigger altered immune profiles, different adjuvants have been used to stimulate different innate immune system players. The studies described in the second aim of this project demonstrate that IDO1 deficient mice are able to mount productive antibody responses upon immunization with model antigens. The extent of the response varied depending upon the adjuvant used. IDO1 ko mice exhibited reduced antibody titers (particularly at early timepoints) in response to immunization using alum and titermax, two particulate adjuvants that lead to indirect activation of DCs and B cells through induction of chemokine production by macrophages and monocytes. In contrast, antibody titers in response to immunization using CFA, an adjuvant that stimulates TLRs to directly activate DCs and B cells, were indistinguishable between IDO1 ko and control C57BL/6 mice. Therefore, these studies suggest that IDO1 plays a role in the induction of serum antibody responses, but that IDO1 is dispensable when B cells receive a secondary stimulus through TLRs.

In summary, the results from this project demonstrate that IDO plays an important role in the development of B cell activation and memory. Previously, IDO was thought to exert a primarily inhibitory effect through dendritic cell-mediated suppression of effector T cells. Our results, demonstrating a second role for IDO in supporting B cell activation and memory, suggest the field will need to redefine IDO as an immune modulatory enzyme rather than an inhibitory enzyme.

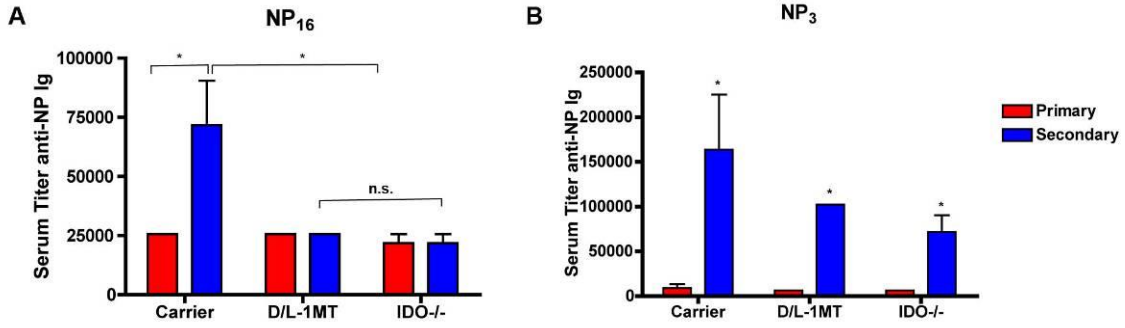

Figure 1. Role of IDO in affinity maturation of secondary immune response. Carrier- and D/L-1MT-treated C57BL/6 mice or untreated IDO1 deficient ($IDO^{-/-}$) mice were immunized with 100μg NP-KLH in alum on day 0. Mice were given a second injection with 100μg NP-KLH in alum 60 days following the first injection. D/L-1MT (400mg/kg, p.o.) and carrier were given b.i.d. for the duration of the experiment. Serum samples were taken 10 days following the first (primary) and second (secondary) immunizations and analyzed for (A) combined high and low affinity and (B) only high affinity anti-NP IgG by ELISA. Data are from a representative experiment of two total with n=5 mice per group. *$p<0.05$, n.s., not significant.

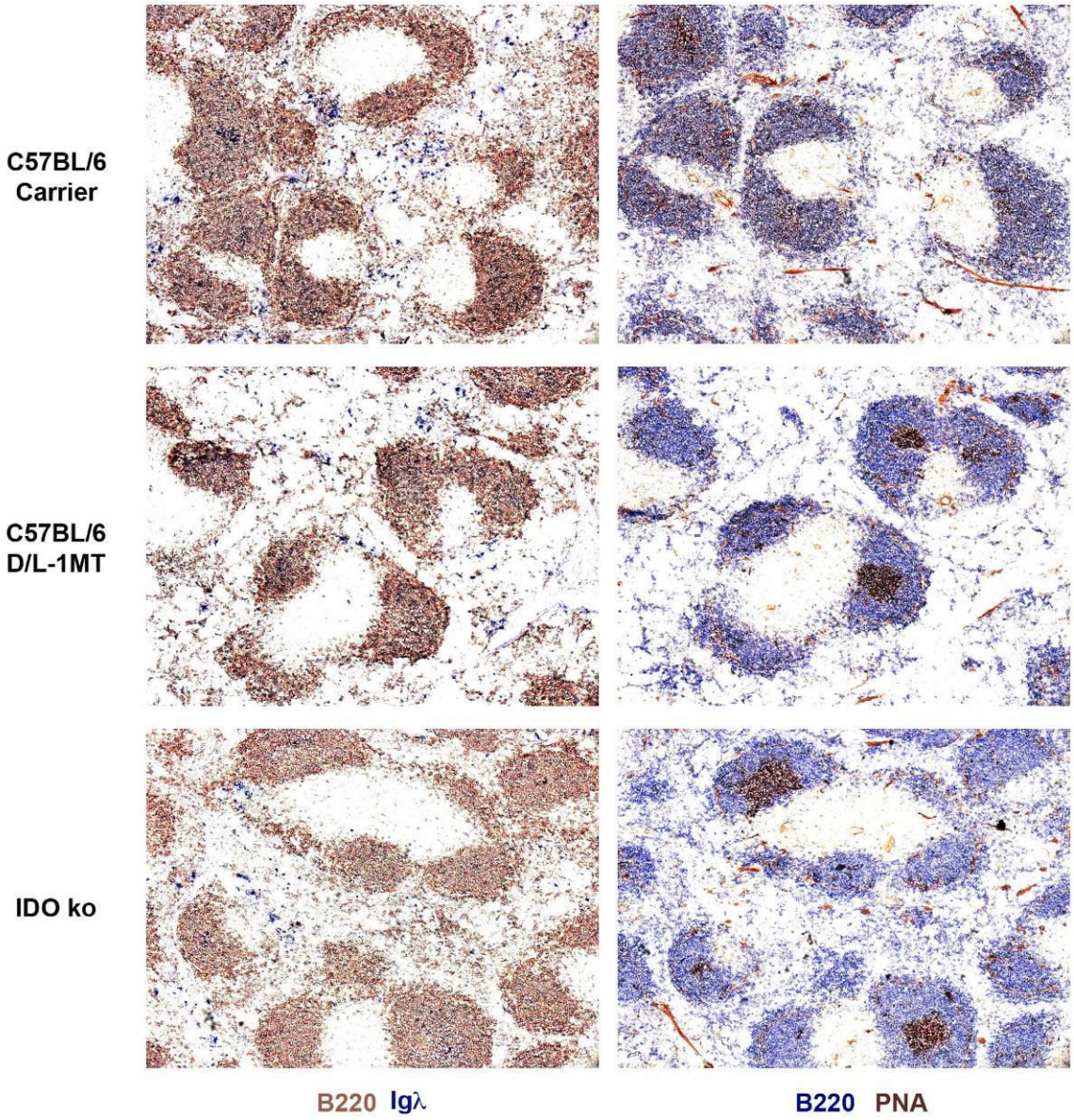

Figure 2. Inhibition of IDO activity alters germinal center response following secondary immunization. Carrier- and D/L-1MT-treated C57BL/6 mice or untreated mice were immunized with 100μg NP-KLH in alum on day 0. Mice were given a second injection with 100μg NP-KLH in alum 60 days following the first injection. D/L-1MT (400mg/kg, p.o.) and carrier were given b.i.d. for the duration of the experiment. Spleens were harvested on day 30 of the second immunization, frozen in OCT, sectioned, and stained with antibodies to B220 and (left) Igλ to identify antibody secreting cells and (right) PNA to identify germinal centers. Shown are representative sections from n=3 mice for each group. Original magnification = 100x.

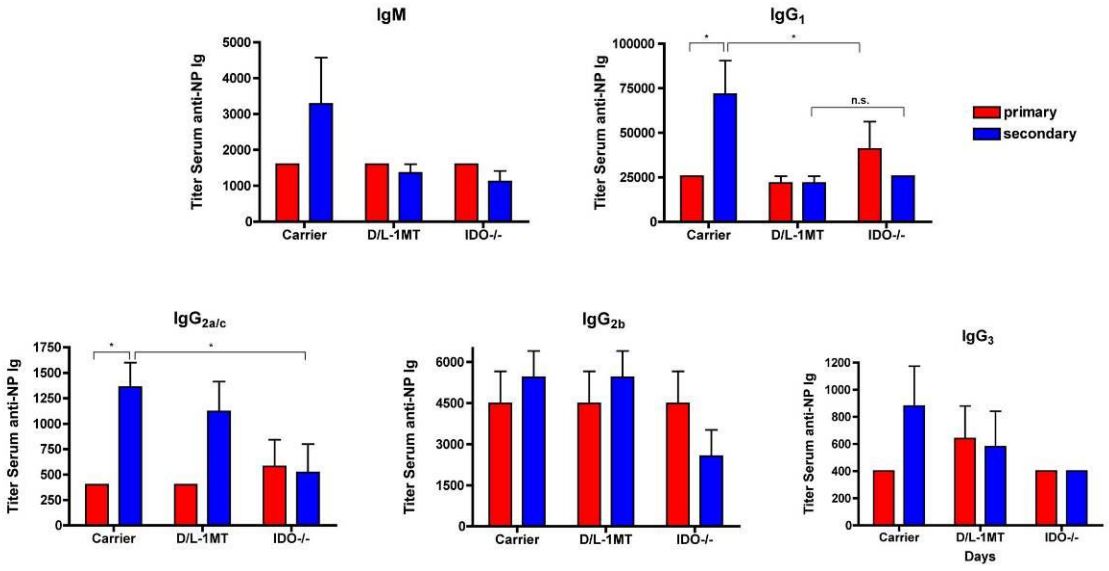

Figure 3. Role of IDO in isotype-switching during memory B cell response. Carrier- and D/L-1MT-treated C57BL/6 mice or untreated IDO1 deficient ($IDO^{-/-}$) mice were immunized with 100μg NP-KLH in alum on day 0. Mice were given a second injection with 100μg NP-KLH in alum 60 days following the first injection. D/L-1MT (400mg/kg, p.o.) and carrier were given b.i.d. for the duration of the experiment. Serum samples were taken 10 days following the first (primary) and second (secondary) immunizations and analyzed for anti-NP IgM, IgG_1, $IgG_{2a/c}$, IgG_{2b}, and IgG_3 by ELISA. Data are from a representative experiment of two total with n=5 mice per group. *$p<0.05$, n.s., not significant.

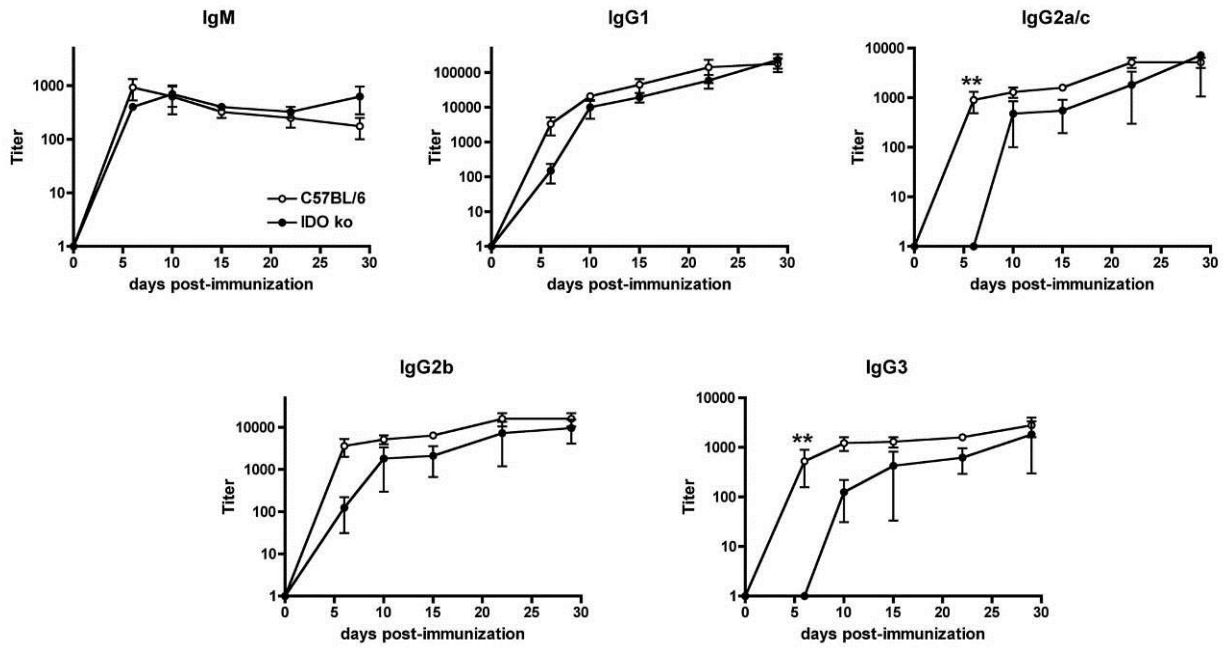

Figure 4. Loss of IDO activity inhibits early B cell responses to NP-KLH in Titermax. C57BL/6 mice and IDO1 deficient (IDO ko) mice were immunized i.p. with 100μg NP-KLH in Titermax on day 0. Serum samples were taken on days 0, 5, 15, 22, and 29 and analyzed for anti-NP IgM, IgG_1, $IgG_{2a/c}$, IgG_{2b}, and IgG_3 by ELISA. n=4 mice per group. **$p<0.05$

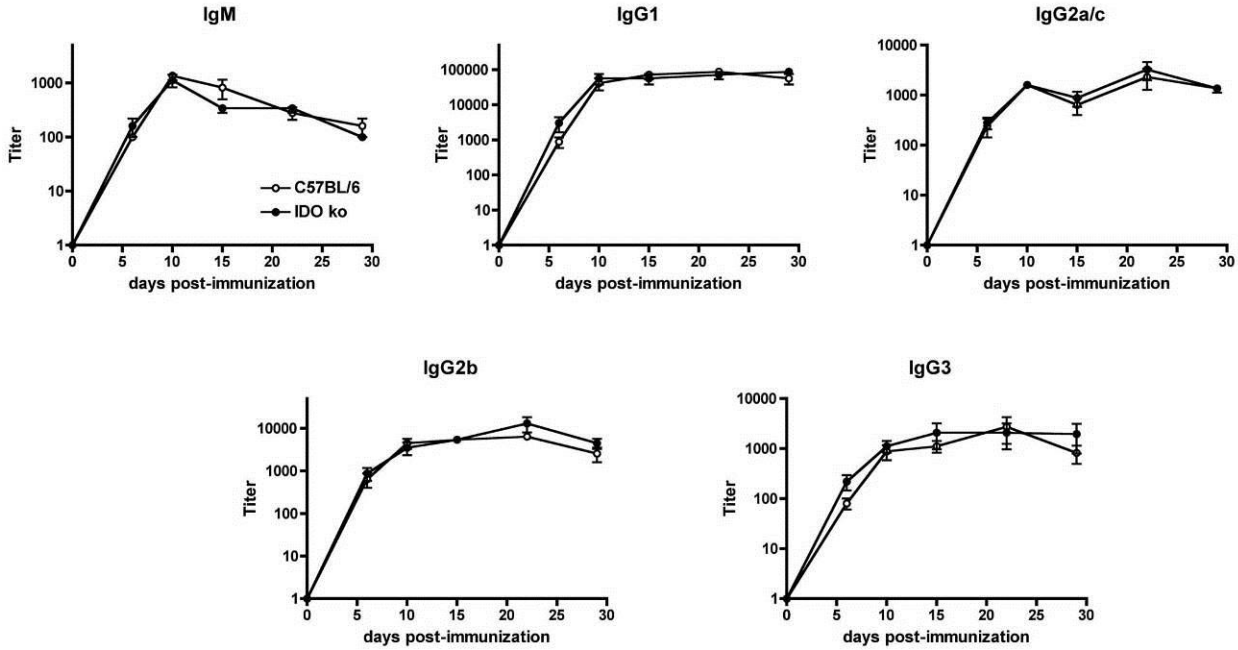

Figure 5. Loss of IDO activity does not inhibit B cell responses to NP-KLH in CFA. C57BL/6 mice and IDO1 deficient (IDO ko) mice were immunized i.p. with 100μg NP-KLH in CFA on day 0. Serum samples were taken on days 0, 5, 15, 22, and 29 and analyzed for anti-NP IgM, IgG_1, $IgG_{2a/c}$, IgG_{2b}, and IgG_3 by ELISA. n=5 mice per group.

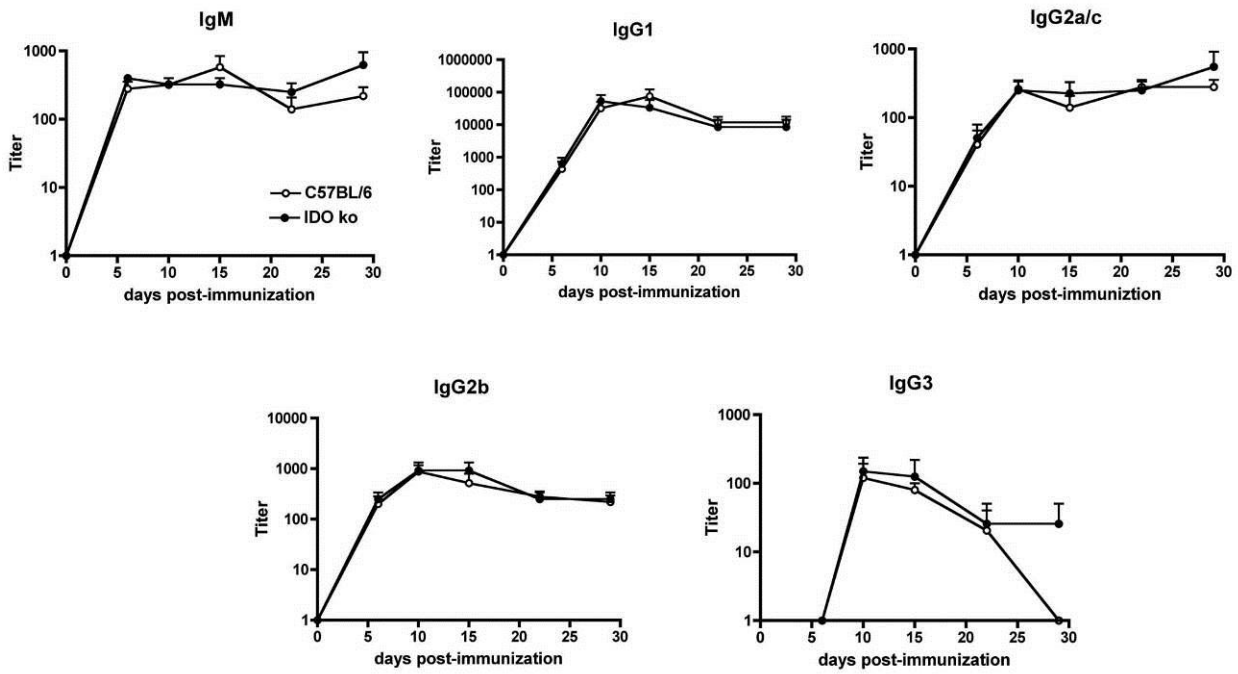

Figure 6. Loss of IDO activity does not inhibit B cell responses to NP-CGG in Alum. C57BL/6 mice and IDO1 deficient (IDO ko) mice were immunized i.p. with 100μg NP-CGG in alum on day 0. Serum samples were taken on days 0, 5, 15, 22, and 29 and analyzed for anti-NP IgM, IgG_1, $IgG_{2a/c}$, IgG_{2b}, and IgG_3 by ELISA. n=5 mice per group.

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__X___No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__X___No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
___X__ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes

_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication, listed in the table, in a PDF version 5.0.5 format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, the number of the publication and an abbreviated research project title. For example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer
Project 3 – Zhang – Publication 2 – Lung Cancer

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1.				☐Submitted ☐Accepted ☐Published
2.				☐Submitted ☐Accepted ☐Published
3.				☐Submitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes___X_____ No_____

If yes, please describe your plans:

The work supported by this grant is part of a larger project defining the role of IDO in B cell-mediated immune responses both *in vitro* and *in vivo*. This project focused on the role of IDO1 in B cell activation and memory in an *in vivo* model antigen system. Ongoing work in the lab is focused on determining the role of IDO1 and a related immunomodulatory enzyme, IDO2, both in *in vitro* and *in vivo* model systems. Once that work is completed, it will be combined with the data generated from this project and submitted to a peer-reviewed journal for publication.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

none

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

none

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___X_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No_____X_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

Laura Mandik-Nayak, PhD

Education

Bucknell University, Lewisburg, PA	B.S.	05/93	Biology
University of Pennsylvania, Philadelphia, PA	Ph.D.	12/99	Immunology
Washington University, St. Louis, MO	Post Doc	12/99 - 03/01	Immunology
Washington University, St. Louis, MO	Post Doc	03/01 - 08/06	Immunology

Positions and Employment

Faculty Appointments:

2006-present Assistant Professor, Lankenau Institute for Medical Research, Wynnewood, PA

2008-present Assistant Professor of Microbiology and Immunology, Jefferson Medical College, Philadelphia, PA

2009-present Adjunct Assistant Professor of Pathology and Laboratory Medicine, Drexel University College of Medicine, Philadelphia, PA

Other Experience and Professional Memberships

Postgraduate Training:

1999-2001 Postdoctoral fellow, Washington University (PI: Dr. David Chaplin), St. Louis, MO

2001-2006 Postdoctoral fellow, Washington University (PI: Dr. Paul Allen), St. Louis, MO

2001- present Member American Association of Immunologists

Honors

1993-1994 N.I.H. training grant (University of Pennsylvania), Philadelphia, PA

1994-1997 N.I.H. training grant (The Wistar Institute), Philadelphia, PA

1994 Gina Finzi Memorial Student Fellowship Award (Lupus Foundation of America)

1997-1998 Dr. Monica H.M. Shander Fellowship Award (The Wistar Institute), Philadelphia, PA

1999 Saul Winegrad Award for Outstanding Dissertation (University of Pennsylvania), Philadelphia, PA

1999-2001 Howard Hughes post-doctoral fellowship (Washington University), St. Louis, MO

2001-2004 N.I.H. post-doctoral training grant (Washington University), St. Louis, MO

2010 Junior Faculty Travel Award (AAI)

Selected Peer-reviewed Publications (Selected from 23 peer-reviewed publications)

1. Mandik-Nayak L., Huang G., Sheehan K.C.F., Erikson J., Chaplin D.D. 2001. Signaling through TNF Receptor p55 in TNF-α-deficient mice alters the CXCL13/CCL19/CCL21 ratio in the spleen and induces the maturation and migration of anergic B cells into the B cell follicle. *J. Immunol*. 167:1920-1928.
2. Mandik-Nayak L., Wipke, B.T., Shih, F.F., Unanue, E.R., and Allen, P.M. 2002. Despite ubiquitous autoantigen expression, arthritogenic autoantibody response initiates in the local lymph node. *Proc. Natl. Acad. Sci. USA*. 99:14368-14373.
3. Mandik-Nayak, L., Racz, J., Sleckman B.P., and Allen, P.M. 2006. Autoreactive marginal zone B cells are spontaneously activated but lymph node B cells require T cell help. *J. Exp. Med.* 203:1985-1998**.**

4. Scott, G.N., J. DuHadaway, E. Pigott, N. Ridge, G.C. Prendergast, A.J. Muller and L. Mandik-Nayak. 2009. The immunoregulatory enzyme IDO paradoxically drives B cell-mediated autoimmunity. *J. Immunol.* 182(12):7509-17.
5. Muller, A.J., DuHadaway, J.B., Chang, M.Y., Ramalingam, A., Sutanto-Ward, E., Boulden, J., Soler, A.P., Mandik-Nayak, L., Gilmour, S.K., and Prendergast, G.P. 2010. Non-hematopoetic expression of IDO is integrally required for inflammatory tumor promotion. *Cancer Immunol. Immunother*., 59:1655-1663.
6. Mandik L., Nguyen K.A., and Erikson J. 1995. Fas receptor expression on B-lineage cells. *Eur. J. Immunol*. 25:3148-3154.
7. Nguyen K.A. Mandik L., Bui A., Kavaler J., Norvell A., Monroe J.G., Roark J.H. and Erikson J. 1997. Characterization of anti-single-stranded DNA B cells in a non-autoimmune background. *J. Immunol*. 159:2633-2644.
8. Mandik-Nayak L., Bui A., Noorchashm H., Eaton A., and Erikson J. 1997. Regulation of anti-double-stranded DNA B cells in nonautoimmune mice: localization to the T-B interface of the splenic follicle. *J. Exp. Med*. 186:1257-1267.
9. Mandik-Nayak L., Seo S.j., Sokol C., Potts K.M., Bui A., and Erikson J. 1999. MRL-*lpr/lpr* mice exhibit a defect in maintaining developmental arrest and follicular exclusion of anti-double-stranded DNA B cells. *J. Exp. Med*. 189:1799-1814.
10. Mandik-Nayak L., Nayak S., Sokol C., Eaton-Bassiri A., Madaio M.P., Caton A.J., and Erikson J. 2000. The origin of anti-nuclear antibodies in bcl-2 transgenic mice. *Int. Immunol.* 12:353-364.
11. Mandik-Nayak L., Seo S.j., Eaton-Bassiri A., Allman D., Hardy R.R., and Erikson J. 2000. Functional consequences of the developmental arrest and follicular exclusion of anti-double-stranded DNA B cells. *J. Immunol*. 164:1161-1168.
12. Seo S., Fields M.L., Buckler J.L., Reed A.J., Mandik-Nayak L., Nish S.A., Noelle R.J., Turka L.A., Finkelman F.D., Caton A.J., and Erikson J. 2002. The Impact of T Helper and T Regulatory Cells on the Regulation of Anti-Double-Stranded DNA B Cells. *Immunity*. 16: 535-546.
13. Shih F.F., Mandik-Nayak L., Wipke B.T., and Allen P.M. 2004. Massive thymic deletion results in systemic autoimmunity through elimination of $CD4^{+}CD25^{+}$ T regulatory cells. *J. Exp.Med.*. 199:323-335.
14. Fusello, A.M., Mandik-Nayak, L., Shih, F.F., Lewis, R.E., Allen, P.M., and Shaw, A.S. 2006. The MAPK scaffold kinase suppressor of Ras is involved in Erk activation by stress and proinflammatory cytokines and induction of arthritis. *J. Immunol.* 177:6152-6158.
15. Wei, G., DeFeo, K., Hayes, C.S., Woster, P.M., Mandik-Nayak, L., Gilmour, S.K. 2008. Elevated ornithine decarboxylase levels activate ataxia telangiectasia mutated-DNA damage signaling in normal keratinocytes. *Cancer Res.* 68(7):2214-22.