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): 01/01/1009 to 12/31/2009**

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 ME Number or SAP Number: 4100047637**

6. **Project Number and Title of Research Project: Project 2, Role of IDO in B Cell-mediated Immunity and Autoimmunity**

7. **Start and End Date of Research Project: 01/01/2009 to 12/31/2009**

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:

   $111,906.67 (DC: $67,862.17, IC: $44,044.50)

   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	Principal Investigator	20%	$22,119.36

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	Research Assistant	10%
Ridge	Graduate Student	50%

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
none		

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 Pathways in Inflammatory Pathogenesis and Treatment of Rheumatoid Arthritis	×NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)	Nov. 2009	$2,062,500 (DC: $1,250,000) IC: $812,500)	$ Not funded
IDO Pathway in the Treatment and Pathogenesis of Rheumatoid Arthritis	☐NIH ☐ Other federal (specify:_____ _____) ×Nonfederal source (specify: Arthritis Foundation)	October 2009	$189,359 (DC: $175,332) IC: $14,027)	$
	☐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:

I have used the preliminary data generated using these research funds to apply for two grants, one from the NIH and one from the Arthritis Foundation. The NIH grant was recently reviewed and received a favorable score. The Arthritis Foundation grant is still pending.

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

The results from aim 1 of the proposal demonstrated that pharmacological inhibition of IDO2, but not IDO1, led to a diminished autoreactive B cell response and attenuated disease course. In the future, we plan to confirm this finding using mice genetically deficient in IDO1 or IDO2. We have also submitted an NIH RO1 proposal and an innovative research grant to the Arthritis Foundation to further explore these results and investigate additional model systems.

Based on the preliminary results obtained in aim 2 of the proposal suggesting a role for IDO1 in driving B cell activation in response to immunization with the model antigen NP-KLH, we plan to do further experiments. These include, confirming the results from the pilot experiment and then expanding our approach to include additional model antigens and adjuvant systems to define the IDO-dependent pathway. Additionally, we will determine if there is also a role for IDO in driving B cell memory. These experiments are being funded by the current cycle of the Pennsylvania Department of Health research grant.

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				
Unknown		1		
Total		**1**		

	Undergraduate	Masters	Pre-doc	Post-doc
White				
Black				
Asian				
Other		1		
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 inflammatory autoimmune disease that affects 3 million Americans. It is characterized by chronic inflammation of the synovial joints, eventually leading to progressive and debilitating destruction of cartilage and bone. Current therapies focus on controlling the symptoms of arthritis, not on the unknown underlying basis for its development. We propose to explore two immunomodulatory enzymes, IDO and IDO2, as disease modifiers of arthritis pathogenesis. Initially associated with protection of the fetus from the maternal immune system, IDO has also been shown to suppress effective immune responsiveness to a number of pathological conditions, including infection and tumorigenesis. However, the role of IDO in classic autoimmune disorders, such as RA, is not well characterized. The conceptualization that IDO acts solely to suppress effector T cell activation has led to the general assumption that inhibiting IDO will exacerbate autoimmune responses. However, recent indications in cancer models suggest a more complex biological role for IDO in actually molding the state of pathological inflammation commonly associated with and contributing to tumorigenesis. If IDO were to contribute similarly to other states of pathological inflammation, inhibiting IDO activity would have the unexpected consequence of ameliorating, rather than exacerbating symptoms. Along these lines, we have obtained preliminary evidence in the spontaneous K/BxN mouse model of RA that inhibiting IDO does indeed interfere with the manifestation of autoimmunity. IDO2, a novel IDO-related enzyme, is less well characterized, but has intriguing features that hint at IDO-related roles in dendritic cell function. In this project, we will use both pharmacological and genetic inhibition of IDO/IDO2 activity in the K/BxN model to determine the role that IDO/IDO2 plays in shaping the autoimmune response leading to arthritis. We suggest that targeting IDO/IDO2 has the exciting potential to lead to a new approach for the prevention and treatment of RA.

Aim 1. Determine which inflammatory features of arthritis induced in K/BxN mice can be ameliorated by targeting IDO/IDO2 activity

Aim 2. Investigate whether IDO directly affects B cell activation or acts indirectly through modulatory effects on the microenvironment.

Aim 1. Determine which inflammatory features of arthritis induced in K/BxN mice can be ameliorated by targeting IDO/IDO2 activity

1MT exerts its inhibitory effect early in the response

To begin to define the mechanism by which 1MT inhibited the initiation phase of arthritis development in K/BxN mice, we determined when 1MT administration was required during the course of arthritis to produce an efficient response. First, we tested whether 1MT was able to inhibit an ongoing arthritic response. K/BxN mice were allowed to develop arthritis and then given 1MT or carrier alone *(Fig. 1a)*. Mice were monitored for arthritis development by measuring joint inflammation by the change in ankle thickness and synovial proliferation and inflammatory cell infiltrates by histology. K/BxN mice that received carrier alone developed severe inflammation in their front and rear paws that began between 28 and 35 days of age. When administered after the onset of inflammation, 1MT had no significant effect on arthritis development, indicating that 1MT needed to be present earlier in the response to have its anti-arthritic effect.

Next, we tested whether treatment with 1MT during the establishment of disease would be sufficient to produce an anti-arthritic effect. To accomplish this, K/BxN mice were given 1MT or carrier alone for 10 days, at which time treatment was stopped and mice were monitored for arthritis development *(Fig. 1b)*. When administered for just this short time period, 1MT was able to significantly attenuate arthritis in the K/BxN mice, demonstrating that blocking IDO activity with 1MT during the initiation phase of the response was sufficient to affect the course of arthritis.

At the termination of the experiment (7wk of age), rear ankles were harvested and examined for histological evidence of arthritis by staining with hematoxylin and eosin *(Fig. 2)*. Carrier-treated mice showed classic signs of arthritis, with a greatly expanded synovium, panus formation, and inflammatory cell infiltrates *(Fig. 2a)*. In contrast, K/BxN mice treated with 1MT prior to *(Fig. 2b)*, but not after *(Fig. 2c)*, the onset of arthritis showed a reduction in the severity of arthritis with minimal synovial expansion and fewer infiltrating inflammatory cells. Together, these data indicate that 1MT is only effective if administered early in the arthritic response, and this short-term exposure is sufficient to attenuate arthritis development even if the treatment is stopped. These data suggest that IDO plays an activating role in establishing the autoimmune profile at the onset of the arthritic response.

IDO2, not IDO1, drives arthritis in K/BxN mice

Our preliminary studies in K/BxN mice demonstrated that treatment with D/L-1MT resulted in a reduced autoantibody response and an attenuated course of arthritis. This treatment inhibits both IDO and IDO2, suggesting that IDO and/or IDO2 normally promote the development of inflammation in this model. To determine if inhibition of IDO1, IDO2, or both was responsible, small molecule inhibitors that target IDO1 or IDO2 (L-1MT and D-1MT, respectively) were used to pharmacologically block IDO activity. K/BxN mice were treated with D-1MT, L-1MT, or carrier alone starting at weaning *(Fig. 3)*. Mice were monitored for arthritis development by measuring joint inflammation by the change in ankle thickness and synovial proliferation and

inflammatory cell infiltrates by histology. K/BxN mice that received carrier alone developed severe inflammation in their front and rear paws that began between 28 and 35 days of age. Administration of D-1MT delayed the onset and attenuated the severity of arthritis. In contrast, L-1MT did not inhibit arthritis, indicating that inhibition of IDO2, not IDO1, alleviates arthritis development in this model.

At the termination of the experiment (7wk of age), rear ankles were harvested and examined for histological evidence of arthritis by staining with hematoxylin and eosin *(Fig. 4)*. Carrier-treated mice showed classic signs of arthritis, with a greatly expanded synovium, panus formation, and inflammatory cell infiltrates. In contrast, K/BxN mice treated with D-1MT, but not L-1MT, showed a reduction in the severity of arthritis with minimal synovial expansion and fewer infiltrating inflammatory cells *(Fig. 4)*. Together, these data suggest that IDO2, not IDO1, is responsible for driving arthritis.

D-1MT, not L-1MT inhibits autoreactive B cell response

Antibodies that recognize the glycolytic enzyme glucose-6-phosphate isomerase (GPI) are the key effector molecules in the disease process in K/BxN mice. Previously, we demonstrated that the reduced arthritis in D/L-1MT-treated mice was the result of a diminished GPI-specific B cell response. To determine if this was affected by inhibition of IDO1, IDO2, or both, K/BxN mice were treated with L-1MT, D-1MT, or carrier alone and followed for serum anti-GPI titers and the number of antibody secreting cells. Titers of anti-GPI Ig *(Fig. 5a)* were significantly lower in D-1MT compared to L-1MT or carrier treated mice. To determine if the reduced serum anti-GPI levels were due to D-1MT treatment affecting the magnitude of the anti-GPI B cell response, the number of antibody secreting cells (ASCs) was quantified in the lymph nodes draining the arthritic joints (dLNs) of L-1MT, D-1MT, or carrier-treated K/BxN mice *(Fig. 5b)*. GPI-reactive ASCs were present in large numbers in the dLNs of L-1MT and carrier-treated K/BxN mice. Numbers of anti-GPI ASCs were significantly reduced in the dLN of D-1MT-treated mice. Together, these data demonstrate that inhibition of IDO2, but not IDO1 activity, diminishes the pathogenic anti-GPI B cell response, resulting in reduced arthritis.

Neither L-1MT nor D-1MT alone is sufficient to alter cytokine response

IDO has been shown to be both an inhibitor of T cell activation and a modifier of the cytokine profile of the resulting T cell response. Our preliminary data showed that K/BxN mice treated with D/L-1MT had reduced levels of the inflammatory cytokines IL-6, MCP-1, and IL-10, while levels of IFNγ and TNFα remained unchanged. To determine if inhibition of IDO1 and/or IDO2 was responsible for this decrease in cytokines, K/BxN mice were treated with L-MT, D-1MT, or carrier alone and monitored for cytokine production. LNs draining the arthritic joints were harvested and stimulated overnight with PMA + ionomycin. Cytokine levels were measured in the culture supernatants by using the flow cytometry-based cytometric bead array assay *(Fig. 6)*. Carrier-treated mice had high levels of IFNγ, TNFα, IL-6, MCP-1, and IL-10. In contrast to the racemic D/L-1MT mix, neither L-1MT nor D-1MT alone affected inflammatory cytokine production *(Fig. 6)*. These data suggest that although inhibition of IDO2 alone is able to alleviate arthritis, inhibition of both IDO1 and IDO2 is required to decrease inflammatory cytokine levels.

Aim 2. Investigate whether IDO directly affects B cell activation or acts indirectly through modulatory effects on the microenvironment.

IDO does not play a direct role in B cell activation in vitro

There are several potential mechanisms by which 1MT could inhibit arthritis development early in the autoimmune response, including affecting the activation of the T and/or B cells responsible for its initiation. In experiments not supported by this grant, we showed that the alleviation of joint inflammation with 1MT was not due to a reduction in T regulatory cells or an altered T helper cell cytokine profile, but resulted from a diminished autoreactive B cell response. Autoantibody secreting B cell numbers were significantly decreased and titers of anti-GPI antibody in the serum were greatly reduced in 1MT-treated K/BxN mice (see preliminary data). This role for IDO in driving B cell responses has not been previously appreciated.

B cells, like most antigen presenting cells, express IDO. Therefore, 1MT could be acting directly on the B cells or inhibiting their activation through an indirect mechanism. To distinguish between these two possibilities, the role of IDO in driving B cell activation and differentiation was measured in purified B cells in vitro *(Fig. 7)*. B cells were assessed for their ability to proliferate, upregulate activation markers (CD25, CD69), upregulate costimulatory markers (CD80, CD86), and differentiate into antibody secreting cells in response to in vitro stimulation. Two approaches were taken to inhibit IDO activity; pharmacologically with D/L-1MT and genetically using B cells from IDO1 deficient (IDO ko) mice. Purified C57BL/6 (IDO1 wild-type) or IDO ko B cells were labeled with the fluorescent dye carboxyfluorescein succinimidyl ester (CFSE) and cultured in vitro with media alone, LPS, or the T cell mimic anti-CD40 + IL-4. Both C57BL/6 and IDO ko B cells proliferated robustly to LPS and anti-CD40 + IL-4, as indicated by a 2-fold decrease in CFSE intensity with each cell division. Similarly, 1MT had no effect on either the number of cells dividing, or the number of cell divisions *(Fig. 7)*.

When B cells become activated, they increase in cell size (blast) and upreguate several cell surface markers, including the early activation markers CD25 and CD69 and the costimulatory molecules CD80 and CD86. These molecules serve both as experimental markers for activation as well as for the B cell's ability to communicate effectively with helper T cells. Upon stimulation with anti-CD40 + IL-4 *(Fig. 8)* or LPS (data not shown), both C57BL/6 and IDO ko B cells increased in cell size and upregulated CD25, CD69, CD80, and CD86. Again, 1MT had no effect on either the increase in cell size or the expression of surface markers of activation.

Finally, the effect of IDO inhibition on antibody secretion and isoype-switching was determined by measuring the amount of IgM and IgG_1 secreted into the culture supernatants *(Fig. 9)*. LPS stimulated B cells to produce IgM, regardless of whether the B cells were from C57BL/6 or IDO ko mice or if the IDO inhibitor, 1MT was present *(Fig. 9a)*. To induce B cells to isotype switch, B cells were cultured with anti-CD40 + IL-4. B cells from all three culture conditions were able to switch the isotype of Ig secreted from IgM to IgG_1, as demonstrated by the decrease in IgM and increase in IgG_1 detected in the culture supernatant *(Fig. 9a,b)*. Therefore, at least in vitro, the lack of IDO activity does not directly affect B cell activation or differentiation. Together,

these data suggest that inhibition of IDO activity affects the pathogenic B cell response in K/BxN mice through an indirect mechanism.

IDO is also required in B cell responses to T cell-dependent model antigens

Our preliminary data demonstrate that IDO is required for the activation and/or differentiation of autoreactive B cells into autoantibody producing cells. These data suggest that IDO may play a more general role in the activation of B cells to non-self antigens. To begin to address the role of IDO on B cell activation, we inhibited IDO activity in mice immunized against the T cell-dependent model antigen, (4-hydroxy-3-nitrophenyl) acetyl - keyhole limpet hemocyanin (NP-KLH) and T cell-independent antigen NP-LPS. IDO activity was inhibited pharmacologically using D/L-1MT and genetically, using IDO1$^{-/-}$ mice. 1MT-treated and carrier-treated C57BL/6 mice and untreated IDO1$^{-/-}$ mice were immunized with 100μg NP-KLH precipitated in alum or 50μg NP-LPS. Anti-NP Ig titers were measured in the serum over the next 4 weeks by ELISA. In response to both NP-KLH and NP-LPS, anti-NP IgG was first detected in carrier-treated C57BL/6 mice at day 5 and levels continued to increase over the next few weeks *(Fig. 10)*. IDO1$^{-/-}$ mice produced reduced levels of anti-NP Ig in response to NP-KLH. After an initial spike at day 5, 1MT-treated C57BL/6 mice also consistently produced lower levels of serum anti-NP IgG *(Fig. 10a)*. In contrast, both IDO1$^{-/-}$ and D/L-1MT treated C57BL/6 mice produced high levels of anti-NP Ig in response to NP-LPS *(Fig. 10b)*. These preliminary data suggest that IDO1 plays a role in the activation of B cells in response to T cell-dependent, but not T cell-independent antigens.

For T cell-dependent stimuli, the anti-NP B cell response can be followed *in situ* as well as in the serum. *In situ*, antibody secreting cell foci can be detected as early as day 2, and reach a peak at 5-8 days post-immunization. At this time, germinal centers begin to appear and remain for several weeks. To determine if 1MT affected the timing or location of antibody secreting cell foci or germinal centers, IDO1$^{-/-}$, carrier-treated, and D/L-1MT-treated C57BL/6 mice were immunized with NP-KLH. At 5 and 10 days after immunization, spleens were harvested, and frozen sections examined for the presence of antibody secreting cell foci and germinal centers. NP-specific B cells were identified by staining for Igλ-expression. Consistent with the reduced serum anti-NP response, both D/L-1MT-treated and IDO1$^{-/-}$ mice appeared to exhibit smaller numbers of antibody secreting cell foci at day 5 and germinal centers at day 10 than carrier-treated C57BL/6 mice *(Fig. 11)*.

Summary and Conclusions

This study supported by the Pennsylvania Department of Health is part of a larger ongoing study (currently supported by institutional funds) to determine the role of indoleamine 2,3-dioxygenase (IDO) in the development and progression of B cell mediated autoimmunity. During the one year period of this proposal, we have completed experiments using the pharmacological inhibitor of IDO, 1-methyl-tryptophan (1MT), and demonstrated that IDO plays an activating role in establishing the autoimmune profile at the onset of the arthritic response. Furthermore, we have found that inhibition of IDO2, but not IDO1, is required for the diminished autoreactive B cell response and amelioration of arthritis. Breeding is underway to generate arthritic mice that are genetically deficient for IDO1 or IDO2 to specifically test the role of each enzyme in the disease

process. In vitro experiments, using both pharmacological inhibition of IDO with 1MT and B cells from IDO1 deficient mice, have also been completed. The results from these experiments suggested that IDO1 does not play a direct role in B cell activation or differentiation. Finally, pilot experiments using an in vivo immunization approach with model antigens and both pharmacological inhibition of IDO with 1MT and IDO1 deficient mice, demonstrated that IDO1 may play an activating role in B cell responses to T cell-dependent, but not T cell independent antigens. Once we have bred enough IDO2 deficient mice, the role IDO2 plays in B cell activation and differentiation both in vitro and in vivo will also be tested. Together, the data generated during this funding period have shown that inhibition of IDO2 at the initial stages of the immune response diminishes the autoreactive B cell response resulting in a reduction of joint inflammation and suggested that IDO may play a more general role in driving B cell responses to both foreign and self antigens.

Scientific Meeting Presentations / Abstracts

These data will be presented at the annual meeting of the American Association of Immunologists in Baltimore, MD (May 7-11, 2010).

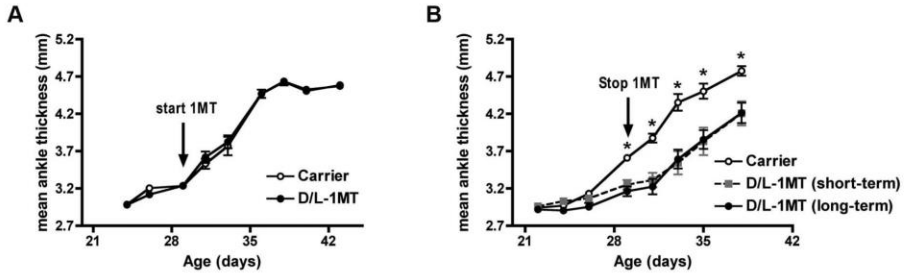

Figure 1. 1MT exerts its effect early in the response. (A) K/BxN mice were allowed to develop arthritis and then were treated with carrier alone or 400 mg/kg 1MT b.i.d. starting at the onset of arthritis (33 days of age). Treatment was continued throughout the remainder of the experiment. (B) K/BxN mice were treated with carrier alone or 400 mg/kg 1MT b.i.d. starting at the age of 21 days. Treatment was either stopped 10 days later (short-term) or continued for the remainder of the experiment (long-term) and the mice followed for arthritis development. Arthritis development was assessed by measurements of rear ankle thickness ± SEM. Representative experiments of 3 total showing n=5 mice for each treatment regimen. ** $p<0.01$.

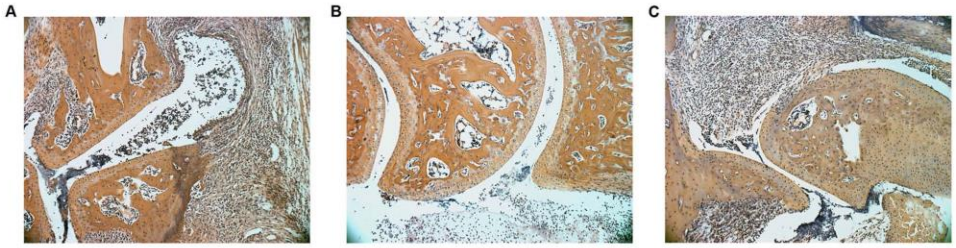

Figure 2. 1MT treated K/BxN mice have reduced joint damage. K/BxN mice were treated with (A) carrier alone or 400mg/kg D/L-1MT b.i.d. by oral gavage starting (B) before the development of arthritis (21 days of age), or (C) after arthritis onset. Metatarsal joint from carrier- or 1MT-treated K/BxN mouse at 42d of age stained with H&E. Original magnification 250X. Representative sections from a total of n=5 mice for each treatment group.

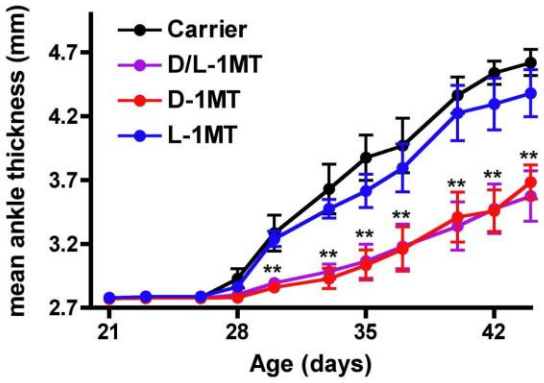

Figure 3. D, not L-1MT inhibits arthritis. K/BxN mice were treated p.o. with 400mg/kg D (red), L (blue), or D/L-1MT (purple) b.i.d. or carrier alone (black) starting at 21 days. Rear ankles were measured as an indication of arthritis and represented as the mean ankle thickness ± SEM. Representative experiment of 3 total, showing n=5 mice for each treatment. **$p<0.01$

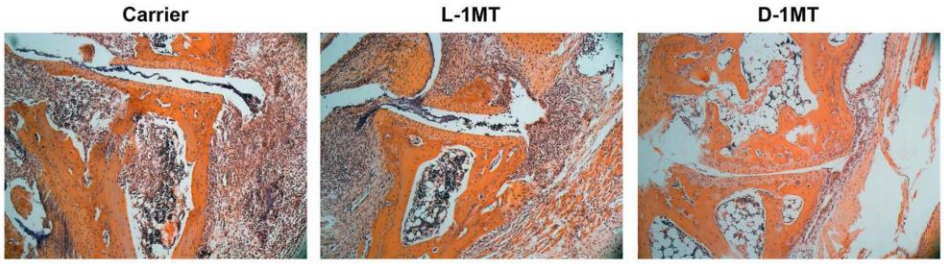

Figure 4. D, not L-1MT inhibits arthritis. Metatarsal joint from D-1MT, L-1MT, or carrier-treated K/BxN mouse at 42d of age stained with H&E. Representative sections from a total of n=5 mice for each treatment group. Original magnification 100X.

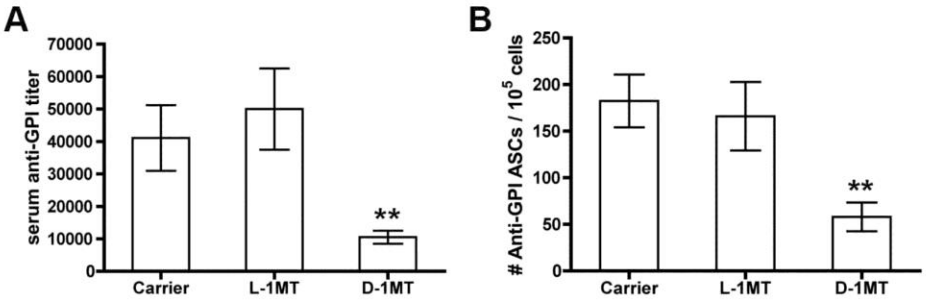

Figure 5. D, not L-1MT inhibits anti-GPI B cell response. K/BxN mice were treated with D-1MT, L-1MT, or carrier alone and analyzed at 6-8 wk of age. (A) The isotype of anti-GPI Ig was measured by ELISA and represented as the mean titer of Ig ± SEM. (B) Antibody Secreting Cells (ASCs) were measured using an ELISpot assay. The graph shows the mean number of ASCs ± SEM in each organ from a representative experiment of 3 total showing n=5 mice for each group. ** $p<0.05$

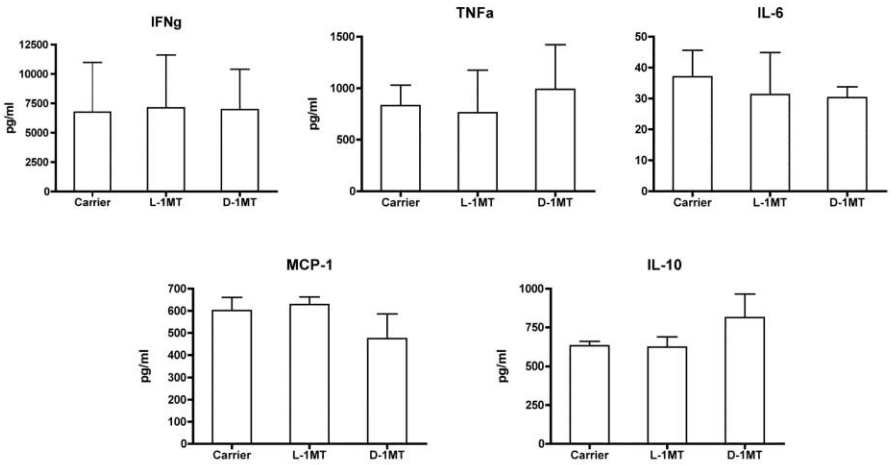

Figure 6. Neither D nor L-1MT treatment alone alters cytokine levels. K/BxN mice were treated with D-1MT, L-1MT, or carrier alone and analyzed at 7-8 wk of age. Cells from the joint dLNs were cultured overnight in PMA (50ng/ml) + Ionomycin (500ng/ml). Cytokines were measured in culture supernatants by cytometric bead array. Graphs show the mean concentration ± SEM from a representative experiment of two with n=5 mice per group.

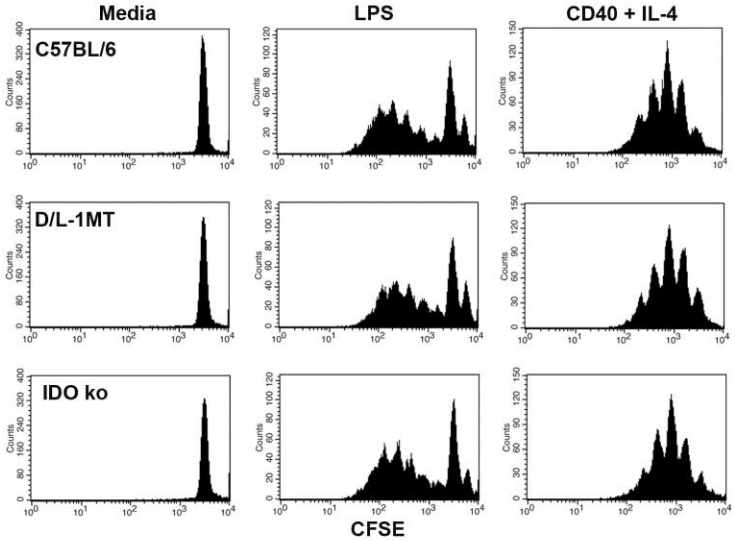

Figure 7. IDO activity is not necessary for B cell proliferation. Purified B cells from C57BL/6 or IDO ko mice were labeled with CFSE and cultured for 3 days in either media alone, 2 μg/ml anti-CD40 + 50ng/ml IL-4, or 25μg/ml LPS. To inhibit IDO activity pharmacologically, B cells from C57BL/6 mice were cultured with 100μM D/L-1MT. Proliferation was measured by flow cytometry as a decrease in CFSE intensity. Histograms show plots representative of 3 separate experiments.

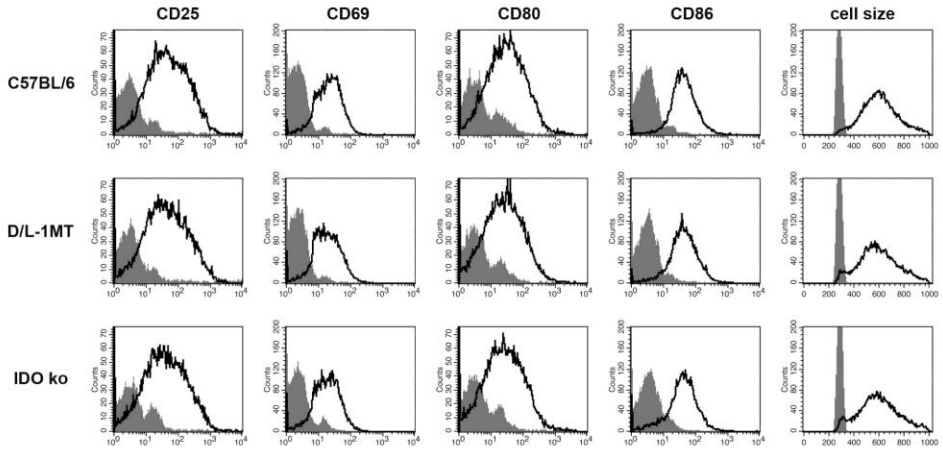

Figure 8. IDO activity is not necessary for upregulation of activation and costimulatory molecules in vitro. Purified B cells from C57BL/6 and IDO ko mice were cultured for 3 days in either media alone or 2 μg/ml anti-CD40 + 50ng/ml IL-4. B cells cultured in 25μg/ml LPS gave similar results. To inhibit IDO activity pharmacologically, B cells from C57BL/6 mice were cultured with 100μM D/L-1MT. Surface expression of CD25, CD69, CD80, and CD86, as well as cell size, was measured by flow cytometry on stimulated B cells (open histograms) overlaid B cells cultured in media alone (filled histograms). This is a representative experiment of 3 total.

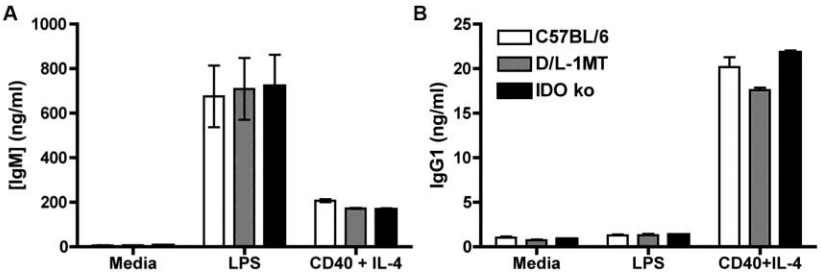

Figure 9. IDO activity is not necessary for production of antibody in vitro. Purified B cells from C57BL/6 and IDO ko mice were cultured for 3 days in either media alone, 2 μg/ml anti-CD40 + 50ng/ml IL-4, or 25μg/ml LPS. To inhibit IDO activity pharmacologically, B cells from C57BL/6 mice were cultured with 100μM D/L-1MT. (A) IgM and (B) IgG_1 secreted into culture supernatants was measured by ELISA. Plots show the mean ± SD of triplicate wells from a representative experiment of 3 total.

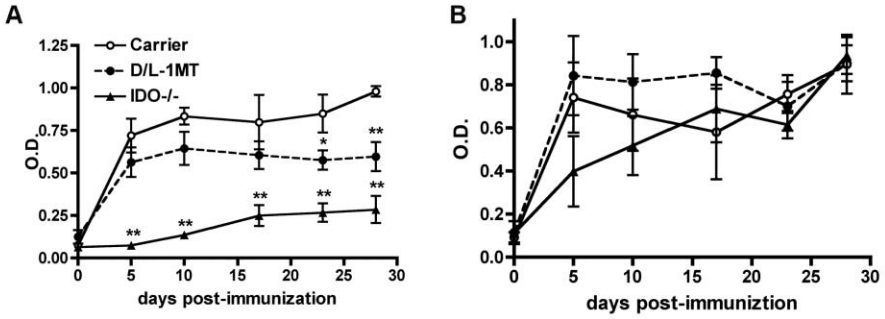

Figure 10. 1MT inhibits B cell response to T-dependent, but not T-independent Ag. Carrier- and D/L-1MT-treated C57BL/6 mice or untreated IDO1$^{-/-}$ mice were immunized with (A) 100μg NP-KLH in alum or 50μg NP-LPS on day 0. D/L-1MT (400mg/kg, p.o.) and carrier were given b.i.d. for the duration of the experiment. Serum samples were taken on days 0, 5. 20, 17, 24, and 28 and analyzed for anti-NP IgG1 by ELISA. Data are from an individual experiment with n=5 mice per group. *$p<0.05$, **$p<0.01$

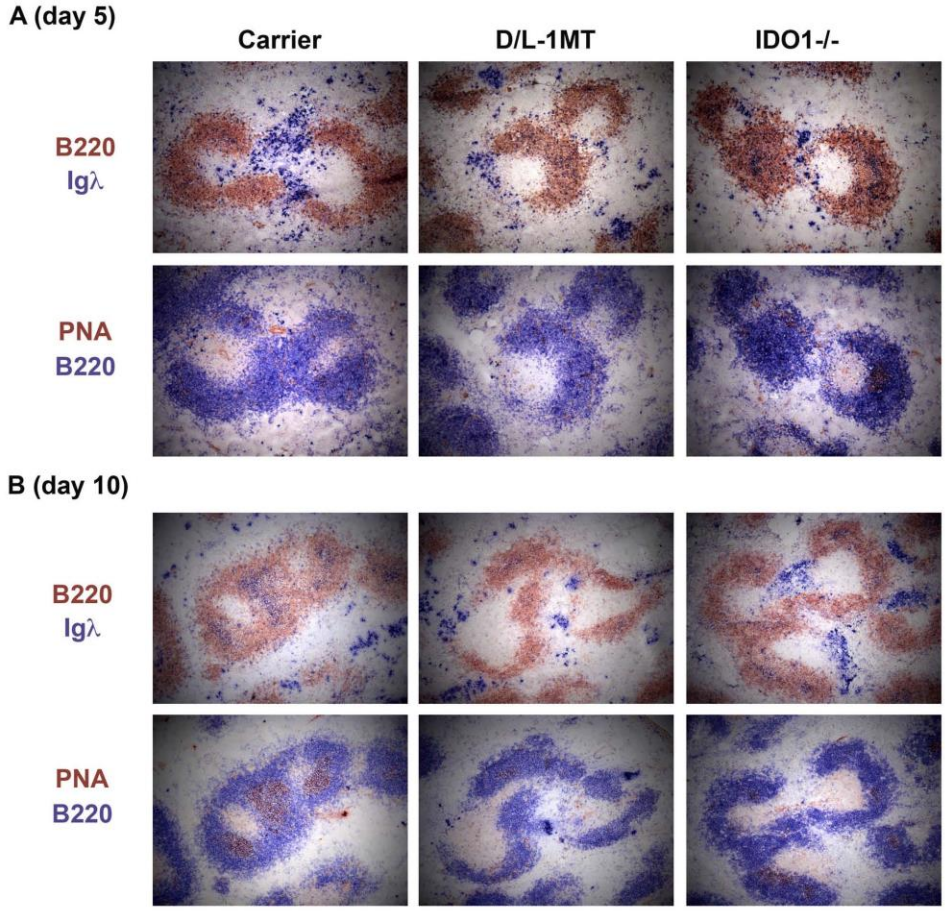

Figure 11. Reduced antibody secreting cells and germinal centers in D/L-1MT and IDO1$^{-/-}$ mice. Carrier- and D/L-1MT-treated C57BL/6 mice or untreated IDO1$^{-/-}$ mice were immunized with 100mg NP-KLH in alum on day 0. D/L-1MT (400mg/kg, p.o.) and carrier were given b.i.d. for the duration of the experiment. Spleens were harvested on days 5 and 10 post-immunization, frozen in OCT, sectioned, and stained with antibodies to B220 and (A) Igl to identify antibody secreting cells and (B) PNA to identify germinal centers. Shown are representative sections from n=5 mice for each group. Original magnification = 100x.

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?

<u>Gender:</u>
_____Males
_____Females
_____Unknown

<u>Ethnicity:</u>
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

<u>Race:</u>
_____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. none				☐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:

Once we have completed experiments evaluating arthritis in the IDO1 and IDO2 deficient mice, we will prepare a manuscript that includes the data generated with this research funding to a peer-reviewed Immunology journal.

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.*

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Laura Mandik-Nayak	Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Bucknell University, Lewisburg, PA	B.S.	1993	Biology
University of Pennsylvania, Philadelphia, PA	Ph.D.	1999	Immunology
Washington University, St. Louis, PA		1999-2001	Immunology
Washington University, St. Louis, PA		2001-2006	Immunology

A. Positions and Honors.

Postgraduate Training:

1999-2001 Postdoctoral fellow, Washington University (PI: Dr. David Chaplin)
2001-2006 Postdoctoral fellow, Washington University (PI: Dr. Paul Allen)

Faculty Appointments:
2006-present Assistant Professor, Lankenau Institute for Medical Research
2008-present Assistant Professor of Microbiology and Immunology, Jefferson Medical College
2009-present Adjunct Assistant Professor of Pathology and Laboratory Medicine, Drexel University College of Medicine

Awards and Honors:
1993-1994 N.I.H. training grant (University of Pennsylvania)
1994-1997 N.I.H. training grant (The Wistar Institute)
1994 Gina Finzi Memorial Student Fellowship Award (Lupus Foundation of America)
1997-1998 Dr. Monica H.M. Shander Fellowship Award (The Wistar Institute)
1999 Saul Winegrad Award for Outstanding Dissertation (University of Pennsylvania)
1999-2001 Howard Hughes post-doctoral fellowship (Washington University)
2001-2004 N.I.H. post-doctoral training grant (Washington University)

Memberships in Professional Scientific Societies:
American Association of Immunologists (member 2001 – present)

B. Selected peer-reviewed publications (in chronological order).
1. Lower T., Henry J., Mandik L., Janosky J., and Friday G.A. Jr. 1993. Compliance with allergen immunotherapy. *Ann. Allergy*. 70:480-482.
2. Norvell A., Mandik L., and Monroe J.G. 1995. Engagement of the antigen-receptor on immature murine B lymphocytes results in death by apoptosis. *J. Immunol*. 154:4404-4413.
3. Roark J.H., Kuntz C.L., Nguyen K.A., Mandik L., Cattermole M., and Erikson J. 1995. B cell selection and allelic exclusion of an anti-DNA Ig transgene in MRL-*lpr/lpr* mice. *J. Immunol*. 154:4444-4455.
4. Mandik L., Nguyen K.A., and Erikson J. 1995. Fas receptor expression on B-lineage cells. *Eur. J. Immunol*. 25:3148-3154.
5. 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.

6. Roark J.H., Bui A., Nguyen K.A., Mandik L., and Erikson J. 1997. Persistence of functionally compromised anti-double-stranded DNA B cells in the periphery of non-autoimmune mice. *Int. Immunol*. 9:1615-1626.
7. 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.
8. Noorchashm H., Bui A., Li H.L., Eaton A., Mandik-Nayak L., Sokol C., Potts K.M., Pure E., and Erikson J. 1999. Characterization of anergic anti-DNA B cells: B cell anergy is a T cell-independent and potentially reversible process. *Int. Immunol*. 11:765-776.
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. Eaton-Bassiri A., Mandik-Nayak L., Seo S.J., Madaio M.P., Cancro M.P., and Erikson J. 2000. Alterations in splenic architecture and the localization of anti-double-stranded DNA B cells in aged mice. *Int. Immunol*. 12:915-926.
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. 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.
13. 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.
14. 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*. 16:535-46.
15. 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.
16. 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.
17. 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.
18. 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**.**
19. 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.
20. Bredemeyer, A.L., Helmink, B.A., Innes, C.L., Calderon, B., McGinnis, L.M., Mahowald, G.K., Gapud, E.J., Walker, L.M., Collins, J.B., Weaver, B.K., Mandik-Nayak, L., Schreiber, R.D., Allen, P.M., May, M.J., Paules, R.S., Bassing C.H., Sleckman, B.P. 2008. DNA double-strand breaks activate a multi-functional genetic program in developing lymphocytes. *Nature.* 456(7223):819-23.
21. Studelska, D.R., Mandik-Nayak, L., Zhou, X., Pan, J., Weiser, P., McDowell, L.M., Lu, H., Liapis, H., Allen, P.M., Shih, F.F., Zhang, L. 2009. High affinity glycosaminoglycan and

autoantigen interaction explains joint specificity in a mouse model of rheumatoid arthritis. *J Biol Chem.* 284(4):2354-62.
22. 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.