Supporting Documentation and Correspondence

Table of Contents

From: **Sesack, Susan R** sesack@pitt.edu
Subject: RE: Plagiarism Concern
Date: April 4, 2025 at 3:37 PM
To: Radjavi radjavi@gmail.com
Cc: Horwitz, Mara horwmx@upmc.edu, Yates, Bill BYATES@pitt.edu, Merz, Nancy J nmerz@pitt.edu

SS

Dear Dr. Radjavi,

We received your allegation of plagiarism on the part of Dr. Daniel Kaplan, Professor of Dermatology in the Medical School, and we conducted a thorough assessment of relevant materials according to our research integrity policy. We have concluded that your concern constitutes a difference of opinion rather than research misconduct. The University has therefore decided to close this case and will not pursue the matter further.

Thank you for bringing this issue to our attention.

Susan

Susan R. Sesack, PhD
Professor of Neuroscience and Psychiatry
Research Integrity Officer
University of Pittsburgh
sesack@pitt.edu

From: **Ali Radjavi** radjavi@gmail.com
Subject: Re: Plagiarism Concern
Date: April 10, 2025 at 10:56 PM
To: Susan R Sesack sesack@pitt.edu
Cc: Mara Horwitz HorwMx@upmc.edu, Bill Yates BYATES@pitt.edu, Nancy J Merz nmerz@pitt.edu

AR

Dear Dr Sesack

Please excuse my delayed reply, which may have more to do with my genuine dislike of this matter, than my schedule.

I submitted my claim of to your office in good faith. I presented evidence that the central, presumably novel, thesis of Dr Kaplans work was identical to one I had previously developed and directly shared with him via email. The content was also publicly available and easily discoverable. If this evidence does not compel your office, I trust you can still understand why it compels me.

Unfortunately, your letter dismisses the matter without explanation or reference to any contrary evidence or rationale. This absence does not address the substance of my claim, nor does it offer a basis for—me or any reasonable party—to reconsider my position.

To ask someone to abandon a documented, good-faith claim without explanation or rebuttal is, in essence, to ask them to be irrational. And I do not believe that is your intent. More likely, your message is that the University is not concerned with what I believe

But I urge you to reconsider that position. I intend to resume efforts to share the documented facts of this matter with other individuals and institutions. Before I do, I once again ask: if the University possesses any information that casts doubt on my claim or that clearly absolves Dr. Kaplan, then you have an ethical obligation to share it. Withholding such information serves no one. It does not protect Dr Kaplan, it does not protect the Universities reputation, and it leaves me with an obligation to pursue the matter based on the only evidence currently available to me. If that evidence leads to a single, logical conclusion, and the University knows otherwise, then failing to share that is a disservice to everyone involved.

Sincerely

Ali

From: **Sesack, Susan R** sesack@pitt.edu
Subject: RE: Plagiarism Concern
Date: April 14, 2025 at 4:10 PM
To: Ali Radjavi radjavi@gmail.com
Cc: Horwitz, Mara horwmx@upmc.edu

SS

Hello Dr. Radjavi,

As indicated in our previous email, we carefully considered your allegation and conducted a thorough assessment as required by our research integrity policy. Our conclusion was that the issue raised was one of professional difference of opinion rather than research misconduct as strictly defined. This conclusion was shared with appropriate University officials who agreed with it. We consider the matter closed, and we will not be responding to any follow-up emails from you.

Sincerely,

Susan

Susan R. Sesack, PhD
Professor of Neuroscience and Psychiatry
Research Integrity Officer
University of Pittsburgh
sesack@pitt.edu

UNIVERSITY of VIRGINIA

Ali Radjavi <ar7bk@virginia.edu>

Itching for immune protection

10 messages

Ali Radjavi <ar7bk@virginia.edu> Sat, Oct 26, 2019 at 4:38 PM
To: dbautista@berkeley.edu, wesley@uw.edu, julian.rayner@sanger.ac.uk, john-harty@uiowa.edu, neil.ferguson@imperial.ac.uk, rogerio.amino@pasteur.fr, ian.cockburn@anu.edu.au, louis.miller@nih.gov, mhoon@dir.nidcr.nih.gov, alan.aderem@seattlechildrens.org, quintana@pasteur.fr, goldberg@borcim.wustl.edu, rahmed@emory.edu, schaerju@hu-berlin.de, Colin.Sutherland@lshtm.ac.uk, smithr@iastate.edu, rogan.lee@sydney.edu.au, peter.atkinson@ucr.edu, psinnis1@jhu.edu, kotsyfakis@paru.cas.cz, peter.krause@yale.edu, mad2256@columbia.edu, Greg.Ebel@colostate.edu, C.S.McKimmie@leeds.ac.uk, volf@cesnet.cz, david.schneider@stanford.edu, gneelaka@odu.edu, pfm0@cdc.gov, linden.hu@tufts.edu, dabente@utmb.edu, sihay@uw.edu, erol.fikrig@yale.edu, gdimopo1@jhu.edu, dwalker@utmb.edu, jfp2@cdc.gov, mdiamond@wustl.edu, jumartin@niaid.nih.gov, piersontc@niaid.nih.gov, jeremy.gray@ucd.ie, aestrada@unizar.es, annetta.zintl@ucd.ie, eharris@berkeley.edu, Michael.muehlenbein@utsa.edu, wbrown@vetmed.wsu.edu, barbet@ufl.edu, swhitehead@niaid.nih.gov, h.hurd@keele.ac.uk, spierce@niaid.nih.gov, stefan.kappe@sbri.org, marc.lecuit@pasteur.fr, YAfshar@mednet.ucla.edu, sweaver@utmb.edu, pagre@jhu.edu, dandrew@jhmi.edu, fzavala1@jhu.edu, sroger@unimelb.edu.au, Douglas.Golenbock@umassmed.edu, martin.schmelz@medma.uni-heidelberg.de, robert.lamotte@yale.edu, irvinea@tcd.ie, dankaplan@pitt.edu, ruslan.medzhitov@yale.edu, sgalli@stanford.edu, isaac_chiu@hms.harvard.edu, soman.abraham@duke.edu, jean.marshall@dal.ca, david.voehringer@uk-erlangen.de, xdong2@jhmi.edu, lhan8@jhmi.edu, goulding@salk.edu, traceyk@northwell.edu, dbroide@ucsd.edu, tilo.biedermann@tum.de, keisuke.nagao@nih.gov, briankim@wustl.edu

Dear Scientists,

I wrote a paper recently called "**The itch-scratch reflex generates protective immunity**" (alternative title: "Why we should scratch our bug bites"). It's a hypothesis paper with one little mouse experiment at the end.

I'm not an expert in the subject matters of this paper, and as of a few years ago I'm a bit of an outsider to the scientific community in general. Regardless, my belief that the central function of itch is being ignored, has continued to nag at me. So I've published this work as an unreviewed preprint, and decided to spam everyone instead of going through the more conventional routes of dissemination (which tend to require institutional backing, and more effort in general)

Perhaps the central hypothesis is incorrect (I don't think it is), or perhaps this point has been made repeatedly since the 1700s (if so, then it hasn't been emphasized enough). Nevertheless, I think this model may have significant value to anyone studying itch, skin allergy/immunity, and arthropod vectored diseases.

here's the link: **https://doi.org/10.1101/808477**

and the abstract:

Itch: its complex neurobiology, its exquisite evolutionary conservation, and even the undeniably euphoric sensation of the scratch it evokes, are all suggestive of a productive physiological function. Nevertheless, we still struggle to answer (or altogether overlook) the basic question of why we itch in the first place. Here, we propose a simple hypothesis: the purpose of itch sensation is to evoke scratching behavior, which in turn boosts protective immunity against the broad range of pathogenic challenges that enter at the skin. We propose that the key function of itch induced scratching is to physically disrupt the skin, serving as a “mechanical adjuvant” that amplifies and directs immune responses to the precise site of potential pathogen entry. As proof of principle, we show that the potent adjuvanticity of itch inducing Compound 48/80 is dependent on this agent’s ability to elicit scratching behavior.
Apologetically yours,

Ali Radjavi PhD

Mail Delivery Subsystem <mailer-daemon@googlemail.com> Sat, Oct 26, 2019 at 4:38 PM

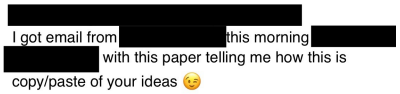
Valda Vinson <vvinson@aaas.org> Mon, Feb 3, 2:40 PM

to me

No I'm afraid I can't do that. It is indeed very challenging to know the provenance of ideas. In this case you had posted the paper on bioRxiv, which is in the public domain.
Sincerely
Valda

Valda Vinson
Executive Editor
Science Journals

On, and shortly after January 31st 2025, I began to receive calls and texts from former colleagues informing me that my work and model had been published by University of Pittsburgh researcher Dr. Dan Kaplan, in the journal *Science,* without attributing my work.



Between 2012-2014, as an immunology graduate student at the University of Virginia, I developed, tested, and presented a scientific model and published a preprint manuscript in 2019 "The itch-scratch reflex generates protective immunity". My due diligence confirmed that I was the first to directly present this model, and the first to provide experimental evidence in support of it.

While Dan Kaplan's paper is tailored to his allergy niche and his preferred mouse models, it is undoubtably based on the same foundational idea I published (see last sentences of Introduction and Conclusion of his Research summary/Structured Abstract in Science.)

I assert that Dr. Kaplan was fully aware of my work, and chose to exclude them from his paper in order to claim originality for its fundamental tenet.

I reject Dr. Kaplans defense that he was unaware of my preprint manuscript, as the paper appears relatively high on Google searches with relevant combination of keywords (see supporting evidence item 3), and because I emailed my BioRx paper to Dan Kaplan in 2019 (item 4).

This is a case study in plagiarism, a clear violation of Upitt Responsible Research Guidelines, misconduct policies at all funding agencies including NIH HHS NSF, and longstanding standard of scientific and academic integrity everywhere.

I have made multiple attempts to confront Dan Kaplan directly prior to initiating these measures. In some of those attempts I employed an angry tone and poor word choice, which may have been misconstrued by Dr. Kaplan. I have made multiple apologies for the misinterpretation, and understand that my former mentor stepped in to assure Dr. Kaplan that this has been a misunderstanding. In spite of apology and outside assurance, Dr. Kaplan has continued to avoid direct dialogue on this matter.

As a result, I have no alternative but to seek investigation by The University of Pittsburgh, and parallel inquiry through the aforementioned institutions and additional private entities.

I ask that your investigation be expedited, and that you provide me with any absolving evidence as quickly as possible.

Please do not hesitate to contact me directly for any clarifications, supporting documentation, or any additional information you need to guide your work.

Ali Radjavi, PhD
radjavi@gmail.com
Cell: 703 216-2973

Supporting Evidence:

1. 2019 BioRx manuscript: https://www.biorxiv.org/content/10.1101/808477v2

2. Dan Kaplan's Science paper, it's and associated coverage:
https://www.science.org/doi/10.1126/science.adn9390

https://www.nature.com/articles/d41586-025-00256-3

3. Google Search Results performed 02.21.25, showing the page and result number of my BioRx paper.

Term (02.21.25)	Page	Result
Itch + Immune + Protective	1	7
Itch + Immune + protective + pdf	1	1
Itch + Scratch + Immune + pdf	1	2
Itch + Scratch + Immune + paper	1	5

4. 2019 email I sent to Dan Kaplan and colleagues with subject line "itching for immune protection"

UNIVERSITY of VIRGINIA

Ali Radjavi <ar7bk@virginia.edu>

Itching for immune protection

10 messages

Ali Radjavi <ar7bk@virginia.edu> Sat, Oct 26, 2019 at 4:38 PM

To: dbautista@berkeley.edu, wesley@uw.edu, julian.rayner@sanger.ac.uk, john-harty@uiowa.edu, neil.ferguson@imperial.ac.uk, rogerio.amino@pasteur.fr, ian.cockburn@anu.edu.au, louis.miller@nih.gov, mhoon@dir.nidcr.nih.gov, alan.aderem@seattlechildrens.org, quintana@pasteur.fr, goldberg@borcim.wustl.edu, rahmed@emory.edu, schaerju@hu-berlin.de, Colin.Sutherland@lshtm.ac.uk, smithr@iastate.edu, rogan.lee@sydney.edu.au, peter.atkinson@ucr.edu, psinnis1@jhu.edu, kotsyfakis@paru.cas.cz, peter.krause@yale.edu, mad2256@columbia.edu, Greg.Ebel@colostate.edu, C.S.McKimmie@leeds.ac.uk, volf@cesnet.cz, david.schneider@stanford.edu, gneelaka@odu.edu, pfm0@cdc.gov, linden.hu@tufts.edu, dabente@utmb.edu, sihay@uw.edu, erol.fikrig@yale.edu, gdimopo1@jhu.edu, dwalker@utmb.edu, jfp2@cdc.gov, mdiamond@wustl.edu, jumartin@niaid.nih.gov, piersontc@niaid.nih.gov, jeremy.gray@ucd.ie, aestrada@unizar.es, annetta.zintl@ucd.ie, eharris@berkeley.edu, Michael.muehlenbein@utsa.edu, wbrown@vetmed.wsu.edu, barbet@ufl.edu, swhitehead@niaid.nih.gov, h.hurd@keele.ac.uk, spierce@niaid.nih.gov, stefan.kappe@sbri.org, marc.lecuit@pasteur.fr, YAfshar@mednet.ucla.edu, sweaver@utmb.edu, pagre@jhu.edu, dandrew@jhmi.edu, fzavala1@jhu.edu, sroger@unimelb.edu.au, Douglas.Golenbock@umassmed.edu, martin.schmelz@medma.uni-heidelberg.de, robert.lamotte@yale.edu, irvinea@tcd.ie, dankaplan@pitt.edu, ruslan.medzhitov@yale.edu, sgalli@stanford.edu, isaac_chiu@hms.harvard.edu, soman.abraham@duke.edu, jean.marshall@dal.ca, david.voehringer@uk-erlangen.de, xdong2@jhmi.edu, lhan8@jhmi.edu, goulding@salk.edu, traceyk@northwell.edu, dbroide@ucsd.edu, tilo.biedermann@tum.de, keisuke.nagao@nih.gov, briankim@wustl.edu

Dear Scientists,

I wrote a paper recently called "**The itch-scratch reflex generates protective immunity**" (alternative title: "Why we should scratch our bug bites"). It's a hypothesis paper with one little mouse experiment at the end.

I'm not an expert in the subject matters of this paper, and as of a few years ago I'm a bit of an outsider to the scientific community in general. Regardless, my belief that the central function of itch is being ignored, has continued to nag at me. So I've published this work as an unreviewed preprint, and decided to spam everyone instead of going through the more conventional routes of dissemination (which tend to require institutional backing, and more effort in general)

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Apologetically yours,

Ali Radjavi PhD

Mail Delivery Subsystem <mailer-daemon@googlemail.com> Sat, Oct 26, 2019 at 4:38 PM

5. 2013-2019 Emails:

This period encompasses the time during which I developed, refined, and conducted experiments related to my work. It also includes the years I spent preparing, editing, and receiving feedback on my manuscript and other related writings. Between two email accounts, there are well over 200 email communications concerning this work. Here, I have included only a select subset to support my claim of discovery. Note I have excluded nearly all emails with my former PI. While those communications are the most personal and organic evidence of my claim, there is sufficient evidence to proceed without them. I will produce those emails if this committee finds it necessary, and after having permission from my former mentor to do so. Below are select excerpts from emailed, published and presented writings:

"While we have some insight into the immune–scratch axis when it is dissregulated, as in allergy, we have never really addressed the normal function of the axis–outside of the pathological context, and prior to disregulation." --From R21 draft

"While the mechanisms of itch sensation are being rapidly elucidated–and the pathological manifestations of itch have been appreciated long since, no satisfactory model has yet been proposed for a normal productive role of itch sensation. Its dedicated neurobiology, its exquisite evolutionary conservation, and even the euphoric sensation in the act of scratching, insinuates a normal and productive function of itch. In this perspective we put forward a simple hypothesis; the purpose of itch sensation is to evoke scratching behavior, which in turn boosts protective immunity against the broad range of pathogenic challenges that start at the skin. We propose that the key function of itch-induced scratching is to physically disrupt the skin barrier, serving as a "mechanical adjuvant" that amplifies and directs immune responses to the precise site of potential pathogen entry." --From Itch paper draft(2)

"We hypothesize that skin scratching has the capacity to boost the immune response to pathogens (e.g. bacteria and viruses) that gain entry to their hosts through the skin. Therefore, scratching may represent a protective response to such infections. The link between skin scratching and the immune system has hitherto not been directly realized. We propose a set of protocols that will allow us to begin to address this connection." --From ACUC Protocol 2013

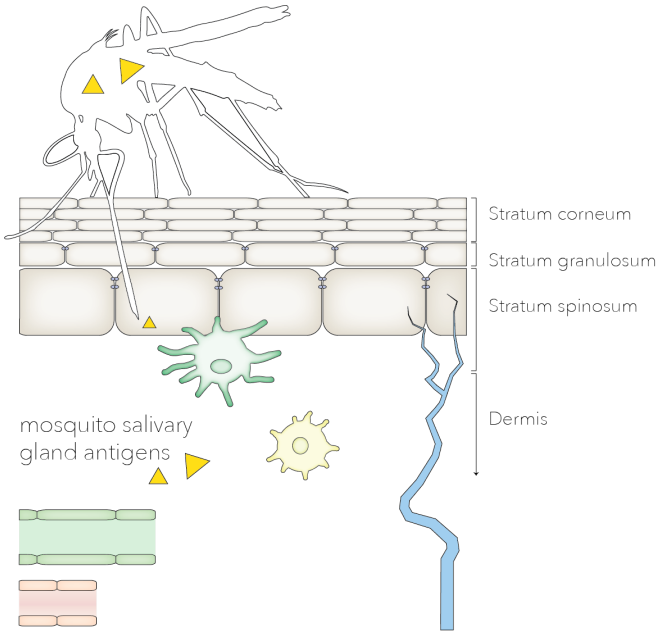
Importantly, the extensive immunomodulatory effects of skin barrier disruption, give rise to numerous points of possible deregulation, making the association between scratching and allergy not a surprising one[23]. Indeed, one hope is that in appreciating the normal physiological function of the itch/scratch reflex we might provide an invaluable context for our understanding of itch related skin and neurological pathologies. --From BioRx

This letter and the model therein are admittedly fun. But the implications of such a system I think are very real. The model enriches our understanding of itch neurobiology by endowing it itch with a central functionality. The model will also help guide our understanding of allergy. By placing hypersensitivity into the context of itch (or more importantly the scratch it evokes), we can begin to view allergic hypersensitivity as a dysregulation of what is a normal and protective function.

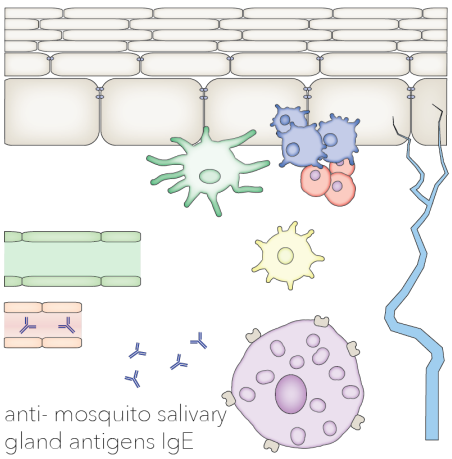
--From "Itching for Immune Protection" 2018 shared draft

6. Raw Data: I have preserved hundreds of GB of data, video, analysis, etc. I will organize and present this data upon request of the investigating committee.

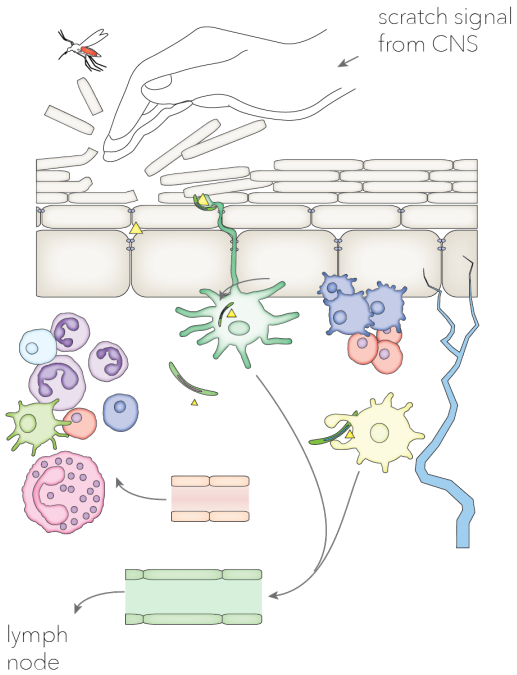
7. 2017 illustrative model of the itch/scratch conferred immune protection in context of mosquito vectored pathogen



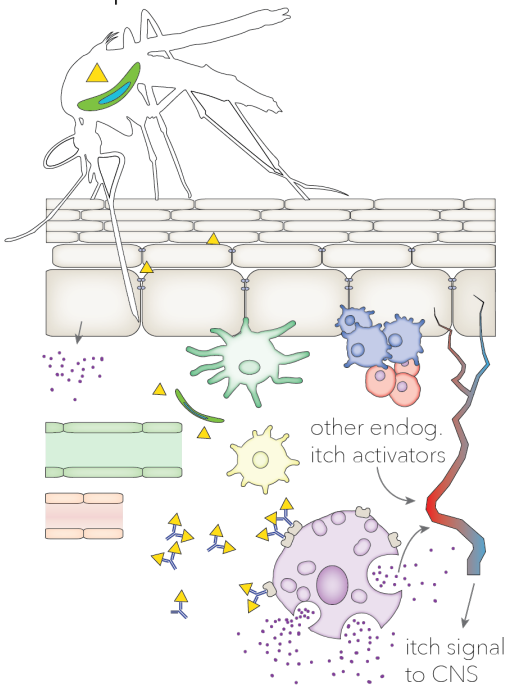
① Naive skin at instance of first bite from uninfected mosquito.



② Altered immune millieu of mosquito bite sensitized skin boosted by repeated bite exposures.



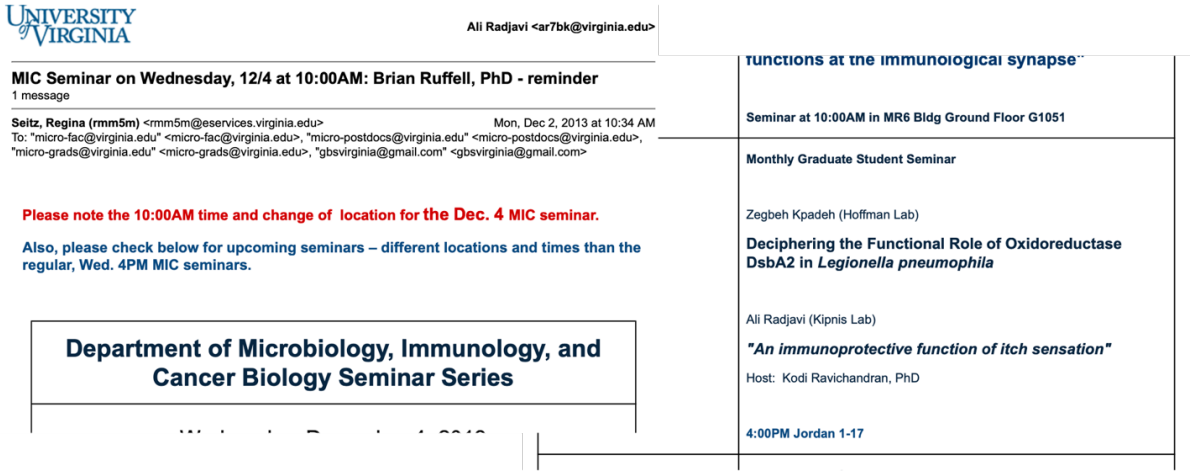
④ Induction of scratching at bite location.



③ Bite from Plasmodium infected mosquito.

Model of immonprotection confereed by mosquito bite induced Itch. 1. The naive skin showing Langerhans cell (green) dermal dentritic cell (yellow) along with itch fiber (blue) and lymphatic (green) and blood (red) vessels. The naive skin is being with the bite of an uninfected mosquito. No itch sensation at this point. **2**. The altered immune millieu of the skin following repeated sensitization with uninfected mosquito bites. Represented here are mosquito salivary gland antigen tissue resident CD8 (blue) and CD4 T cells, Fc receptor decorated mast cell (purple), and circulating anti-mosquito salivary antigen IgEs. The itch/scratch reflex to mosquito bites has been established at this point **3**. Skin challenge with bite of Plasmodium infected mosquito. Itch fiber activation through crosslinking of mosquito salivary antigens/IgE complex, as well as other sources of endogenous itch activators. Itch signal is transmitted to CNS. **4**. Scratching reflex leads to skin barrier disruption, upregulation of Langerhans/dermal dendritic cell antigen uptake functions and migrtion to the draining lymph node. Skin barrier disruption leads to recruitment of Neutrophils, pDcs, ILcs, CD4s,CD8s and eusonophils to site of skin disruption.

8. 2013 UVA Graduate Student Seminar



UNIVERSITY of VIRGINIA

Ali Radjavi <ar7bk@virginia.edu>

MIC Seminar on Wednesday, 12/4 at 10:00AM: Brian Ruffell, PhD - reminder
1 message

Seitz, Regina (rmm5m) <rmm5m@eservices.virginia.edu> Mon, Dec 2, 2013 at 10:34 AM
To: "micro-fac@virginia.edu" <micro-fac@virginia.edu>, "micro-postdocs@virginia.edu" <micro-postdocs@virginia.edu>, "micro-grads@virginia.edu" <micro-grads@virginia.edu>, "gbsvirginia@gmail.com" <gbsvirginia@gmail.com>

Please note the 10:00AM time and change of location for the Dec. 4 MIC seminar.

Also, please check below for upcoming seminars – different locations and times than the regular, Wed. 4PM MIC seminars.

Department of Microbiology, Immunology, and Cancer Biology Seminar Series

functions at the immunological synapse"

Seminar at 10:00AM in MR6 Bldg Ground Floor G1051

Monthly Graduate Student Seminar

Zegbeh Kpadeh (Hoffman Lab)

Deciphering the Functional Role of Oxidoreductase DsbA2 in *Legionella pneumophila*

Ali Radjavi (Kipnis Lab)

"An immunoprotective function of itch sensation"

Host: Kodi Ravichandran, PhD

4:00PM Jordan 1-17

9. 2013 R21 draft with emphasis on vector-borne disease w/ faculty edits

Abstract:

Vector-borne diseases, including malaria, are a significant source of morbidity and mortality worldwide. One commonality with these infections is the delivery of pathogens though an insect bite that induces an itch-scratch reflex. A large body of work from allergy and contact hypersensitivity studies, have demonstrated many ways by which superficial injury to the skin from scratching, can change downstream immunological outcomes [1-5]. While allergy provides insight into the immune–scratch axis during dysregulation, we have never truly addressed the normal function of the axis without pathology. Here we seek to test the hypothesis that skin scratching is a protective host reflex. Building on preliminary data showing skin scratching at the site of antigen challenge to be a potent immunomodulator, we hypothesize that the scratch response to bites from *Plasmodium*-carrying mosquitos is a immunologically-relevant protective function of the itch scratch reflex. Independent of our protective hypothesis, we challenge mice intradermally with *Plasmodium chabaudi*, and ask how the acute itch/scratch reflex might change the nature and magnitude of the immune response to infection. Could this impact vaccine development? Why would this matter to the NIH—they will not give a shit about the conservation of the itch/scratch reflex.

Specific Aim #1: How does disruption of the epidermal skin layer by scratching can modulate the host immune response to intradermal plasmodium infection? Provide rationale. Restate hypothesis. Write full sentences. 10^3 *Plasmodium chabaudi* sporozoites are injected intradermally into C57/B6 mice, followed by repeated manual abrasion of the injection site intended to mimic physiological scratching frequency. Blood is assessed for cytokines, post infection tissue samples are analyzed for readouts of adaptive and innate immunity. What may these results show? What would the impact be?

Specific Aim #2: *To test for a protective function of skin scratching in the Plasmodium chabaudi model of murine malaria.* How is this different from Aim 1? While several studies, along with our own preliminary data, show that mechanical disruption of the skin activates and amplifies the local immune response to antigen, it remains unknown whether these responses have a protective function in the context of a live pathogen. Due to its substantial biological characterization, clinical relevance, and potent itch/scratch induction, the mosquito bite represents an ideal model in which to test for a protective capacity of the itch/scratch reflex. We aim to show that the normal physiological itch/scratch reflex to mosquito bites, can promote protection against reexposure to *Plasmodium chabaudi* delivered by mosquito bite.

Significance: How many people are dying? What don't we understand about the immune response? How can the findings from this proposal help treat human disease?

The importance of the itch/scratch reflex is suggested by both its conservation throughout the animal kingdom and by the euphoric reward associated with the act of scratching. The words itch and scratch evoke a litany of pathologies from eczema, to allergy, to drug induced itch. So deep rooted is our association of itch with pathology that common wisdom warns us not to scratch our insect bites, and even when we inevitably give in to the urge, the euphoria scratching elicits can be a guilty one. Yet the itch/scratch reflex remains exquisitely conserved in evolution, and even the euphoric reward of scratching behavior seems to suggest that there may be an underlying purpose to itch and the scratching reflex it evokes. This reads like a blog post. What about people dying of malaria?

Though experimentally untested, it has been suggested that scratching may function to physically remove antigen from the skin (citations!). However, this model could only operate in cutaneous antigen challenge, as biting insects deposit the bulk of salivary content below the epidermal reach of scratching(Refs). Even in the context of cutaneous antigen exposure, the benefit of physically removing antigen by scratching may be outweighed by the risk of spreading antigen deeper into the tissue and laterally to uninfected skin areas. A second hypothesis that scratching may operate to physically remove a biting pest from the skin is challenged by knowledge that itch sensation usually develops long after the feeding insect has left the bite area, 10-15 minutes post bite in the case of mosquitos(Refs), and 2-6h in tick bites(Refs). In short, beyond anecdotally evidenced hypotheses whose? , no protective function of the itch scratch reflex has been demonstrated. This is also not a discussion section.

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With a ubiquitous distribution of pathogen infected biting insects that leave a non-feeling bite radius of just a few microns on the nearly $2m^2$ surface of human skin, any mechanism that focuses and amplifies the immune response to pathogen deposited at the insult site would be tremendously advantageous. Several published reports intimate the connection between disruption of skin tissue and inflammation. In a model reported by Ng et al scratching the mouse ear with a needle tip evokes neutrophil influx along the swath of the wound within 15 minutes of injury[2] there is a Science paper by Nathan Peters and David Sacks showing how needle stick and insect bite cause this! Tim Lammerman also has a couple papers examining needle stick responses in skin. In tape stripping models, (which may be achieving the same outcome as scratch), disruption of the stratum corneum by repeated tape strips stimulates the sampling of antigen by dendritic projections of langerhan cells[3]. Perhaps most interestingly the remarkable efficacy of Jenner's original smallpox vaccine came not from the immunogenicity of the strain as previously thought, but from his method of inoculation–scarification of the skin[4]. These findings, together with our own preliminary data make a case for a model in which physical abrasion of the skin can evoke immune upregulation at the site of tissue disruption and potential pathogen entry.

In the case of malaria, the profound importance of the early skin stage of infection has long been masked in models that infect mice intravenously with Plasmodium infected red blood cells because these studies aim to induce and characterize CMI. Whereas the direct blood route achieves fulminant cerebral malaria in susceptible mice, the intradermal skin route of infection delivered through the mosquito vector appears to be immunologically protective[7, 8]. The complex behaviors of *Plasmodium* at the skin stage have only recently come to light[8-10]. Sporozoites from the mosquito salivary gland are deposited in the dermis of the skin, followed by migration to blood vessels which can continue up to 2h post deposition[8, 11, 12]. Protective antigen-specific CD8 T cells are primed in the lymph nodes that drain the skin bite[8]. Importantly, many antigens are conserved between the developmental stages of *Plasmodium*[13], making anti-sporozoite immunity protective against subsequent lifecycle stages[8]. Collectively, the skin stage of malaria infection may represent a short window of opportunity for immune activation prior to infection of the liver —an intrinsically immunosuppressive organ where plasmodium is effectively cloaked from immunosurvailance[14-16]. Perhaps you should focus on the fact that the only effective vaccine for malaria is the irradiated sporozoite vaccine. This supports your hypothesis that the deposition of parasites in this stage of the life cycle induces protective immunity—your goal is to make these responses stronger.

The emerging immunological significance (citations) of the skin stage of *Plasmodium* infection, taken together with the immune activating outcome of mechanical disruption of the stratums corneum, lends a curious attractiveness to the hypothesis that scratch may be operating as a "mechanical adjuvant", boosting the local immune response and conferring immunoprotection.

Appreciating the normal homeostatic function of the itch/scratch response can provide an invaluable context for our understanding of itch related skin pathologies. Moreover, with an incalculable number of mosquito, tick, mite and sandfly bites a year, public knowledge of a robust protective function of the scratch reflex can confer, meaningful and cost-free protection against blood-borne pathogens carried by parasitic arthropods. Furthermore, the study will elucidate the basic immune effects of scratching, which will be valuable wheather that immune modulation is benign or as we hypothesize, protective.

Innovation:

To our knowledge, viewing the itch/scratch reflex as a protective immune activator has not been reported. Moreover, no study that we are aware of has directly tested the immunological outcome of skin scratching to live pathogen, and few studies have meaningfully address the immunomodulatory properties of skin scratching to any antigen outside the context of allergy.

Preliminary data in support of general hypothesis:

Itch inducing compounds have immune activating properties that are dependent on scratching.

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We injected OTII mice intradermally, on the left shoulder with ovalbumin, alone or in combination with either of two itch inducing compounds. OTII mice have a nearly monoclonal T cell repertoire directed against chicken egg ovalbumin. 24h later we harvested the draining brachial lymph nodes, and stained their single cell suspensions for CD4 T cell marker and CD69 early activation marker. Both itching compounds chloroquine (histamine dependent) and compound 48/80, potentiated CD69 expression on T cells in OTII mice. When OTII mice are intradermally challenged with ovalbumin, T cell activation can be measured. In WT mice challenged with ovalbumin, CD69 on T cells was not measurable, owing to the low abundance of naturally occurring ovalbumin reactive T cells. (the clonal abundance for any given antigen is estimated at around 50 per mouse(Ref), far beyond the practical limit of detection) It is being done. See papers by Marion Pepper and Mark Jenkins. Importantly, this adjuvant-like property of both itch inducers was not limited to CD4 T cells (including which important cells?)in OTII mice. WT mice similarly challenged with ovalbumin and chloroquine, showed higher titers of anti-ova IgG1 and ovalbumin plus compound 48/80, showed higher titers of ovalbumin-specific IgG1 and IgG2b.

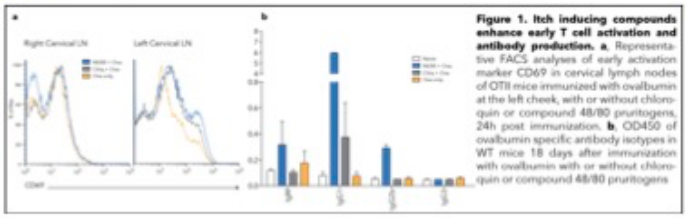

Figure 1. Itch inducing compounds enhance early T cell activation and antibody production. a, Representative FACS analyses of early activation marker CD69 in cervical lymph nodes of OTII mice immunized with ovalbumin at the left cheek, with or without chloroquin or compound 48/80 pruritogens, 24h post immunization. **b**, OD450 of ovalbumin specific antibody isotypes in WT mice 18 days after immunization with ovalbumin with or without chloroquin or compound 48/80 pruritogens

To prove that aduvant like properties of itch inducers is derived from their elicitation of the scratching behavior, scratching was physically blocked. We developed custom jackets with a plastic guard that effectively protected against scratching at one shoulder while permitting scratching at the other. Inhibiting scratching at the site of ovalbumin injection, effectively blocked T cell activation assessed by CD69 expression and anti-ova IgG1 activating property of itch inducer compound 4880 (Figure 2?), demonstrating that the adjuvanicity of compound 4880 is derived from its ability to induce scratching. Stats are needed here. Make the data as compelling as possible. An identical loss of effect was observed when ovalbumin+ compound 4880 was administered at the left cheek and scratching was inhibited using a coned Elizabethan collar (data not shown?).

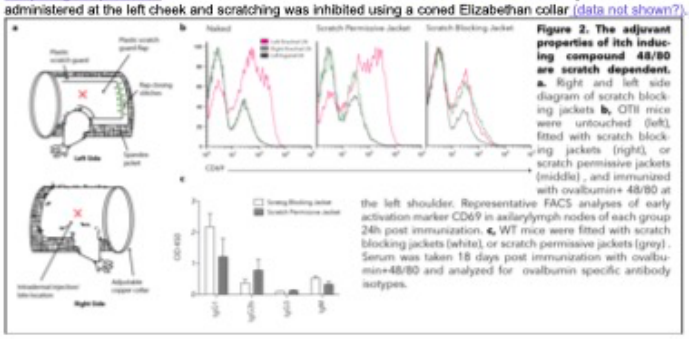

Figure 2. The adjuvant properties of itch inducing compound 48/80 are scratch dependent. a, Right and left side diagram of scratch blocking jackets **b**, OTII mice were untouched (left), fitted with scratch blocking jackets (right), or scratch permissive jackets (middle) , and immunized with ovalbumin+ 48/80 at the left shoulder. Representative FACS analyses of early activation marker CD69 in axillarylymph nodes of each group 24h post immunization. **c**, WT mice were fitted with scratch blocking jackets (white), or scratch permissive jackets (grey) . Serum was taken 18 days post immunization with ovalbumin+48/80 and analyzed for ovalbumin specific antibody isotypes.

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Specific Aim#1 How does the immunomodulatory properties of skin scratching modify the host response to *Plasmodium chabaudi*

Rational: Insect bites matter. People are dying. The immunological consequence of scratching has not been addressed in the context of infection with a live pathogen. In Aim 1 we seek to understand how the innate and adaptive arms of the immune system are changed by skin scratching at the site of intradermal plasmodium challenge.

Our preliminary data shows that T cell activation is enhanced when mice are allowed to access to a vaccine inoculation site. Following *Plasmodium* infection, we anticipate that scratch will again result in heightened amplitude of the immune response, including T cell, myeloid cell activation, and Ab production. While mechanical disruption of the skin activates and amplifies the local immune response to model antigen, it is also expected that the skew of the CD4 T helper response will be affected. Thus, the immunological skewing resulting from disruption of the stratum corneum towards TH1/TH2/TH17 responses will be tested.

SA 1a Experiment 1: How does scratch affect adaptive immunity: antigen specificity, T cell activation, T cell cytokine profile, Ab isotype. Our own preliminary data shows striking differences between CD69 activation in response to 30 manual scratch passes administered at once, and the same number scratch passes given over a 12h interval (exp needs repeating before figure). Therefore, mice will be manually scratched 10m, 15m, 20m, 40m, 1h, 3,h 8h, 16h, 24h, 36h post Plasmodium sporozoite challenge to approximately recapitulate the physiological frequency of scratch bouts (is this published?), which from our preliminary data appears to be a critical checkpoint for CD69 activation. Plasmodium Chabaudi sporozoites will be purified as previously described[22]. Every two days post infection, blood samples will be taken for luminex arrays to determine plasma concentrations of IFN-g, TNF-a, IL-6, IL-10, and IL-4 and *Plasmodium* specific Ig isotypes will be quantified by ELISA. Single cell suspensions of spleens and draining lymph nodes will be split into three aliquots and stained with either CD3,CD4,CD8, CD44, CD62L, CD69, CD19 (lymphoid numbers), IFNg, IL-2, IL-10, IL-4 (lymphoid phenotype panel), or CD3, CD19, NK1.1,Ly6-C, Ly6-G, CD11 (myeloid numbers) for FACS analysis. Don't give the panel, just say numbers and phenotypes of lymphocytes and myeloid cells.

SA 1bExperiment 2: How are innate Immune parameters affected by scratch: Langerhans activation, neutrophilic influx and APC function. 8 week old male C57BL/6 mice will be infected intradermally at the left shoulder with *Plasmodium chabaudi*, with or without mechanical scratching as described in Specific Aim 1a. At 20m, 1h, 4h, 12h, 24h, and 48h timepoints a 1cm punch biopsy of depilated skin surrounding the injection and control site will be processed for confocal microscopy and flow cytometry. For flow cytometry experiments single cell suspensions from digested skin will be stained with CD45, Ly6C, Ly6G, CD11b, CD11c, CD3 and MHCII, to allow for determination of neutrophil, macrophage, and lymphocyte influx into infected skin that has been manually scratched or sham treated. Confocal staining panels will include Langerin, CD11c and MHCII, to allow for visualization of the Langerhan and dermal dendritic cell network 1cm from the epicenter of the injection site, for total cell number and morphology to determine efflux of resident cells from the tissue. What might this tell you? What do you expect?

SA 1cTo test scratch dependent changes in antigen presentation efficiency we will infect WT mice concomitantly with Plamsodium and with DQ-Red OVA (this is not infectious) (a reporter for phagocytic uptake that fluoresces in the low pH of the lysosomal compartment of APCs), and subject mice to the same manual scratch paradigm as in Experiment 1. 10m, 1h, 4h, 12h, 24h, and 48h we will subject the shaved 1cm punch biopsy of the injection site to confocal microscopy to detect phagocytic uptake of OVA by DQ-Red fluorescence. To more directly test APC function, we will collect brachial lymph nodes draining the injection site at 4, 12, 24 and 48h post injection. Single cell suspensions of lymph nodes will be seeded onto 24-well plates and co-cultured with CFSE labeled CD4 T cells of OTII mice. 24h later wells will be assayed for T cell proliferation via FACS analysis of CFSE dilution, and the supernatants assayed for IL-2 secretion via ELISA. The major assumption in the aforementioned assays is that ova is a surrogate for plasmodium antigen. To directly test presentation of plasmodium antigens we will perform the same co-culture experiments (measuring CFSE dilution and IL-2 secretion) with cultured polyclonal T cells derived from Ly5.1 mice immunized with plasmodium homogenates in CFA. I'm not crazy about this last part.

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10. 2013 ACUC protocol with scientific justification

Animal Care & Use Committee

UNIVERSITY of VIRGINIA

Principal Investigator: ***JONATHAN KIPNIS***
PI Department: ***Md-Nesc Neuroscience***
Protocol Title: ***The effect of scratching on immune system***
Protocol Number: ***Copy of 3923, J718104 (Full Edit)***
Protocol Submittal Type: ***1st or 2nd annual review - with modifications***

Cover Letter provided by person who submitted protocol

Date letter (protocol) was submitted: 02/28/2013
Letter was submitted by: RADJAVI, ALI (ar7bk)
This is the First Year Annual Renewal. Changes since last submition have included:

Removed: "ear scratching" experiments and subsequent 2-photon from the protocol

Added: A BSL1 model of plasmodium chabaudi infection in "procedure 3"

SUMMARY OF SPECIES PROCEDURES

Species Procedure # 1: **Exp 1. Brush Scratching**
Species: **Mice**
Animal Handler(s):
AFSHAR, KYANA (ka2un) - Health Status: **OK FOR WORK** , Must return by: 12/10/13 (982-3859)
RADJAVI, ALI (ar7bk) - Health Status: **OK FOR WORK** , Must return by: 03/30/13 (982-3859)
SMIRNOV, IGOR (is4b) - Health Status: **OK FOR WORK** , Must return by: 08/07/13 (982-3859)

Species Procedure # 2: **Exp. 2 Prevention of Scratching**
Species: **Mice**
Animal Handler(s):
AFSHAR, KYANA (ka2un) - Health Status: **OK FOR WORK** , Must return by: 12/10/13 (982-3859)
RADJAVI, ALI (ar7bk) - Health Status: **OK FOR WORK** , Must return by: 03/30/13 (982-3859)
SMIRNOV, IGOR (is4b) - Health Status: **OK FOR WORK** , Must return by: 08/07/13 (982-3859)

Species Procedure # 3: **Exp. 3 Plasmodium chabaudi infection model**
Species: **Mice**
Animal Handler(s):
AFSHAR, KYANA (ka2un) - Health Status: **OK FOR WORK** , Must return by: 12/10/13 (982-3859)
RADJAVI, ALI (ar7bk) - Health Status: **OK FOR WORK** , Must return by: 03/30/13 (982-3859)
SMIRNOV, IGOR (is4b) - Health Status: **OK FOR WORK** , Must return by: 08/07/13 (982-3859)

Principal Investigator: **KIPNIS, JONATHAN** Dept: **MEDICINE-NEUROSCIENCE DEPT** PO Box: **801392** E-Mail: **jk3pv@virginia.edu** Work Phone: **434-964-658** Fax: **434-982-4380** Home Phone: **434-823-5726**	Contact Person: **RADJAVI, ALI** Dept: **UNKNOWN** PO Box: **UNKNOWN** E-Mail: **ar7bk@virginia.edu** Work Phone: **982-3859** Fax: **434-982-4380** Home Phone: **703-216-2973**

Protocol Abstract:

We hypothesize that skin scratching has the capacity to boost the immune response to pathogens (e.g. bacteria and viruses) that gain entry to their hosts through the skin. Therefore, scratching may represent a protective response to such infections. The link between skin scratching and the immune system has hitherto not been directly realized. We propose a set of protocols that will allow us to begin to address this connection.

Protocol Identification: PILOT STUDY

Transgenic Animals: NO

Molecular Imaging Core: NO

Radioactive Materials: NO

Biological Agents: NO

Hazardous Chemicals: NO

Patient Care Areas: NO

DEA Controlled Substances: YES **NOTE:** DEA Controlled Substances must be securely locked in a manner that cannot be easily removed, and up-to date usage logs (including inventory, amount used and balance remaining) must be maintained.

Location(s) of DEA Controlled Substances:
MR-4: **6127**

Current Funding Source	Status	Dates	Description	Grant Numbers

UVA DEPARTMENTAL	**ACTIVE**	**stat up funds. unrestricted.**	N/A	N/A

Animal Use Justification:

Why must animals be used: There is no way to recapitulate this model in vitro as the system depends on complex interactions of various organ systems that can only be found in living animals, specifically interactions between the skin, the immune system, central nervous system (CNS), and peripheral nervous system (PNS). Second, the research requires a behavioral response in a living animal (i.e. skin scratching). In addition, the model requires the pharmacological induction of pruritis, which is a complex phenomenon that requires a live animal.
Why is the species you've selected the most appropriate for this research protocol? Mus Musculus is the most appropriate species for this research protocol, as knockout mice that would be used in the project only exist in mice (most immediately the gastrin-releasing peptide receptor knockout mouse)

Alternatives to the Use of Animals:

Replacement: As described in the animal use justification there is no replacement for a live animal model in our system. Reduction: The number of animals proposed for this study represents the minimum number of mice needed for a statistically meaningful pilot study. Refinement: Many experimental protocols were proposed, considered and discarded before arriving at the current protocol, which we believe is one that addresses our scientific question while minimizing pain and distress to the mice .
Non-duplication searches – the hits retrieved might not have suggested a boost to immune response but were they at all related to your proposed study and if so, in what manner?

Survey of the Literature for NON-DUPLICATION SEARCH # 1	**Survey of the Literature for NON-DUPLICATION SEARCH # 2**
Database or source consulted: **MEDLINE** Date search was performed: **02/29/2012** Years covered by the search: **70** TO **12** Keywords: **scratch AND immune response** Summarize your findings:	Database or source consulted: **WEB OF SCIENCE** Date search was performed: **02/29/2012** Years covered by the search: **70** TO **12** Keywords: **scratch AND immune response** Summarize your findings:
42 hits returned non of which suggests that scratch would boost the immune response	103 hits returned, and as above, none of these wrks suggests that scratch would serve a booster to the immune system.
Has this study been conducted previously? **NO**	Has this study been conducted previously? **NO**

SPECIES PROCEDURE # 1

Species: **Mice**
Procedure ID: **Exp 1. Brush Scratching**
Does this Species Procedure (in part or in whole) contain a Breeding Colony? **No**

Justify the Number of Animals Needed:

based on our previous experience with other animal models, we expect that 3 mice per group will give us sufficient preliminary data:
1. Brush Scratching; 3mice/group X2 groups X4 timepoints = 24 mice

USDA Pain and Distress Category:
B: **0** Animals/Year
C: **0** Animals/Year
D: **24** Animals/Year
E: **0** Animals/Year

Anticipated maximum number of animals per year: **24**

Alternatives to Painful and Distressful Procedures SEARCH # 1	**Alternatives to Painful and Distressful Procedures SEARCH # 2**
Database or source consulted: **MEDLINE** Date search was performed: **02/28/2013** Years covered by the search: **70** TO **13** Keywords: **scratch AND immune response** Summarize your findings:	Database or source consulted: **WEB OF SCIENCE** Date search was performed: **02/28/2013** Years covered by the search: **82** TO **13** Keywords: **scratch immune response** Summarize your findings:
43 hits returned none of which suggests that scratch would boost the immune response	145 hits returned none of which suggests that scratch would boost the immune response
Did you find a less painful/less distressful alternative that could accomplish the goals of your animal use protocol? **NO**	Did you find a less painful/less distressful alternative that could accomplish the goals of your animal use protocol? **NO**

Where will the Animals in this Species Procedure be Housed?
MR4 Vivarium

Animal Husbandry (Mice): STANDARD HUSBANDRY

Does the nature of the research require an exemption for Social Housing? NO

Does the nature of the research require an exemption for Environmental Enrichment? NO

Live Animal Work Outside of Vivarium: YES

Location(s) of Live Animal Work:
MR-4: **6127**

Satellite Housing (Mice): NO

Will any SURGERY (Survival and/or Non-Survival) be performed on animals? YES

Location(s) where animal surgeries are performed:
MR-4: **G043C**

- SURVIVAL SURGERY? **YES**
- NON-SURVIVAL SURGERY? **YES**

Pharmaceutical(s) Used: (Mice)

Pre-Anesthetic:
NONE SELECTED

Inductional Anesthetic:
Isoflurane to Effect

Maintenance Anesthetic:
Isoflurane to Effect

PRIMARY Post-procedural analgesic:
NONE SELECTED

SECONDARY Post-procedural analgesic:
NONE SELECTED

Euthanasia Agent:
Euthanasia Solution IP or IV

Does the nature of the research require the use of Non-Pharmaceutical Grade Drugs? NO

Will guillotines be used on any LIVE animals in this procedure? NO

Multiple Major Survival Surgical Procedures (MMSS): NO

Tumor Generation: NO

Will any *conscious (unanesthetized)* animals be kept in a restraint device during the course of these experiments for more than momentary procedures (longer than 5 minutes)? NO

Main Procedure Description:

Procedures, which require Humane Endpoints and Criteria for Euthanasia, to be performed on animals used in this procedure section:

- NONE

Protocol 1: The first model is derived from Yamaoka et al1. Mice are anesthetized to with Isoflurane. Using hair clippers, a 3cm2 region of fur is lightly clipped on the back of the mouse, with care taken not to make direct contact between the skin and clippers. Scratching is conducted manually on 2cm2 dorsal surface using a hard bristle toothbrush at a scratching frequency of 90 times/minute for in 30 second scratch/rest cycles lasting 4 minutes (2 minutes total scratching). Scratching pressure will be monitored by a push/pull force gauge. The brush is attached to the hook of the gauge and suspend over the anesthetized mouse with the bristles making slight contact with the mouse's skin at a force approaching zero. Scratching force is monitored on the gauge and is maintained near .98N. Alternatively a 25g weight adhered to the backside of the toothbrush and scratching is conducted by moving the brush by the tip of the handle only along the plane of the dorsal surface. After the scratching interval, mice are allowed to recover from anesthesia and returned to their home cage. At intervals 5 minutes, 30 minutes, 2 hours and 6 hours after returning to their home cages, mice will be injected with a lethal dose of Nembutal (5mg/mouse), perfused with heparinized PBS, and skin biopsied for inflammatory infiltrates via immunohistochemistry.

Post-Procedural Details:

This procedure is relatively mild (it is meant to recapitulate a physiological level and degree of scratching) so we do not anticipate pain or distress (which would add an unwanted variable to our experiments as well, given that pain inhibits itch). The animals will be monitored for up to five minutes post procedure before being returned to their home cages Nevertheless we will employ the ACUC Policy on Recognition and Assessment of Pain, Stress, and Distress of Laboratory Animals in this observation period.

Procedural Training:

Ali Radjavi

Animal Handler Responsibilities

AFSHAR, KYANA (ka2un)
ACUC Defined General Requirements
UVa - Orientation Seminar: **01/08/2013**
UVa - Working Safely with Animals Training: **11/27/2012**
UVa - Animal Facility Rules and Procedures Training: *Required before entering any vivarium at UVa, provided in-person by the vivarium supervisors, unique for each vivarium at UVa.*
LATA - Mouse Training: **11/27/2012**
LATA - Anesthesia and Analgesia of Rodents: **11/28/2012**
Biomethodology (restraint, blood collection, gavage, and/or injection)

RADJAVI, ALI (ar7bk)
ACUC Defined General Requirements
UVa - Orientation Seminar: **03/06/2008**
UVa - Working Safely with Animals Training: **03/06/2008**
UVa - Animal Facility Rules and Procedures Training: **05/15/2012**
LATA - Mouse Training: **01/22/2008**
LATA - Anesthesia and Analgesia of Rodents: **01/22/2008**
Biomethodology (restraint, blood collection, gavage, and/or injection)
Critical Clinical Monitoring - Non-surgical (Humane Endpoints)
Euthanasia
Post-Surgical Care

Supervise and/or Train Others
Survival Surgery
UVa - Rodent Survival Surgery Training: **01/28/2008**

SMIRNOV, IGOR (is4b)
ACUC Defined General Requirements
UVa - Orientation Seminar: **10/06/2010**
UVa - Working Safely with Animals Training: **09/09/2010**
UVa - Animal Facility Rules and Procedures Training: **09/15/2010**
LATA - Mouse Training: **09/09/2010**
LATA - Anesthesia and Analgesia of Rodents: **09/09/2010**
Biomethodology (restraint, blood collection, gavage, and/or injection)
Critical Clinical Monitoring - Non-surgical (Humane Endpoints)
Euthanasia
Post-Surgical Care
Supervise and/or Train Others
Survival Surgery
UVa - Rodent Survival Surgery Training: **09/09/2010**

SPECIES PROCEDURE # 2

Species: **Mice**
Procedure ID: **Exp. 2 Prevention of Scratching**
Does this Species Procedure (in part or in whole) contain a Breeding Colony? **No**

Justify the Number of Animals Needed:

Based on our previous experience with other animal models, we expect that 3 mice per each group will give us sufficient preliminary data. 3mice/groupX2 groupsX2 itch drugs X2 repeats= 24

USDA Pain and Distress Category:
B: **0** Animals/Year
C: **0** Animals/Year
D: **24** Animals/Year
E: **0** Animals/Year

Anticipated maximum number of animals per year: **24**

Alternatives to Painful and Distressful Procedures SEARCH # 1	**Alternatives to Painful and Distressful Procedures SEARCH # 2**
Database or source consulted: **MEDLINE** Date search was performed: **02/28/2013** Years covered by the search: **70** TO **13** Keywords: **Scratch AND Immune response** Summarize your findings:	Database or source consulted: **WEB OF SCIENCE** Date search was performed: **02/28/2013** Years covered by the search: **82** TO **13** Keywords: **scratch AND immune response** Summarize your findings:
43 hits returned none of which suggests that scratch would boost the immune response	145 hits returned none of which suggests that scratch would boost the immune response
Did you find a less painful/less distressful alternative that could accomplish the goals of your animal use protocol? **NO**	Did you find a less painful/less distressful alternative that could accomplish the goals of your animal use protocol? **NO**

Where will the Animals in this Species Procedure be Housed?
MR4 Vivarium

Animal Husbandry (Mice): STANDARD HUSBANDRY

Does the nature of the research require an exemption for Social Housing? NO

Does the nature of the research require an exemption for Environmental Enrichment? NO

Live Animal Work Outside of Vivarium: YES

Location(s) of Live Animal Work:
MR-4: **6127**

Satellite Housing (Mice): NO

Will any SURGERY (Survival and/or Non-Survival) be performed on animals? YES

Location(s) where animal surgeries are performed:
MR-4: **G043C**

- SURVIVAL SURGERY? **YES**
- NON-SURVIVAL SURGERY? **NO**

Pharmaceutical(s) Used: (Mice)

Pre-Anesthetic:
NONE SELECTED

Inductional Anesthetic:
Isoflurane to Effect

Maintenance Anesthetic:
Isoflurane to Effect

PRIMARY Post-procedural analgesic:

NONE SELECTED

SECONDARY Post-procedural analgesic:
NONE SELECTED

Euthanasia Agent:
NONE SELECTED

Does the nature of the research require the use of Non-Pharmaceutical Grade Drugs? YES

If YES, describe the compound, the justification for its use, how many animals will receive it, what groups of animals will receive it, at what dosage and for how long. If a pharmaceutical grade alternative is available, explain why it cannot be used. How is the non-pharmaceutical compound made? How do you ensure its sterility and stability?

Compound 48/8- is not available pharmaceutical grade. It induces an acute histamine dependent itch. . Animals will recieve 100ug comound 48/80 in a 2.5uL intradermal injection. The itch disipates within 90minutes at this dosage. The compound is made by Sigma through the condensation of N-methyl-p- methoxyphenethylamine with formaldehyde. This formulation has been extensively used in the literature. It is aliquoted in the cell culture hood and reconstituted with sterile saline.

Will guillotines be used on any LIVE animals in this procedure? NO

Multiple Major Survival Surgical Procedures (MMSS): NO

Tumor Generation: NO

Will any *conscious (unanesthetized)* animals be kept in a restraint device during the course of these experiments for more than momentary procedures (longer than 5 minutes)? NO

Main Procedure Description:

Procedures, which require Humane Endpoints and Criteria for Euthanasia, to be performed on animals used in this procedure section:

- NONE

We wish to generate a novel model that induces a cutaneous itch sensation but inhibits the mouse's ability to scratch the affected area. While the itch induction protocols are well established, physically precluding the scratch reflex for an extended period of time (5 days in our model) is to our knowledge untested. To avoid complications arising from the effect of muscle disuse, we wish to avoid any methodology that restrains the mouse in a way that inhibits muscle use and motility. To this end, we will use Either: a. commercially available mouse jackets (Harvard Apparatus) or b. elizabethan collars (Kent Scientific) to restrict the mouse's ability to scratch an epidermal injection site that is covered by the jacket or protected by the cone (for controls, the mouse jacket will be modified to allow access to the injection site). Mice will be fitted 1 day prior to the experiment initiation to acclimate the mice to the jacket. To fit the jacket or collar, mice are anesthetized with Isoflurane. After testing for complete anesthesia with foot pinch, the jacket is fitted. Mice will be monitored daily until the end of the experiment to ensure proper fit and integrity of the jacket/collar. After five days the mice are anesthetized with Isoflurane, after testing for complete anesthesia with foot pinch, the jacket/collar is removed and mice are injected subcutaneously at the left flank for jackets or left cheek for collars, with the following protocol: Itching is induced by the subcutaneous injection of one of two established pruritogenic agents along with ovalbumin peptide. Mice are anesthetized with Isoflurane. After testing for complete anesthesia with foot pinch, 100ug of compound 48/803 or 200ug Chloroquine4 is injected along with 40ug ovalbumin subcutaneously at the left flank behind the neck of the mouse. At these concentrations of pruritogenic agents, scratching behavior begins immediately after, and dissipates to baseline within 90 minutes after administration. Immediately after injections, the jacket/collar is refitted and the mouse is placed individually into a clear acrylic box in which scratching behavior is recorded by camera for a period of 2 hours. After this period the mouse is returned to its home cage. Blood (50uL) is collected on days 0, 5 and1 0 via tail vein nick and mice are euthanized at day 10 post injection with a lethal dose of Nembutal (5mg/mouse), and tissue is taken for subsequent analysis.

Post-Procedural Details:

All mice will be monitored daily from the time when the jacket/collar is fitted until the end of the experiment to ensure proper fit and integrity. After administration of pruritogenic agents, the animals will be recorded by video for 2 hours, with video monitored continuously in real-time for the first 10 minutes, and then every five minutes after that.

Although it has been shown that the effects of the pruritogenic agents proposed in this protocol subside within 90 minutes, it is still possible that over the next 8 days mice fitted with control jackets (that allow access to the injection site) may scratch the site to the point of wounding. Any wound, or physical breach of the skin would introduce unwanted variables to our experimental system, and compromise the validity of interpretations. Therefore, if bleeding or wounding is observed at the injection site, the animal will be euthanized. If wounding becomes more than an isolated incident, then mice given "scratchable" control jackets will be switched to the protective jacket 24 hours after injection.

Procedural Training:

Ali Radjavi

Animal Handler Responsibilities

AFSHAR, KYANA (ka2un)
ACUC Defined General Requirements
UVa - Orientation Seminar: **01/08/2013**
UVa - Working Safely with Animals Training: **11/27/2012**
UVa - Animal Facility Rules and Procedures Training: *Required before entering any vivarium at UVa, provided in-person by the vivarium supervisors, unique for each vivarium at UVa.*
LATA - Mouse Training: **11/27/2012**
LATA - Anesthesia and Analgesia of Rodents: **11/28/2012**
Biomethodology (restraint, blood collection, gavage, and/or injection)

RADJAVI, ALI (ar7bk)
ACUC Defined General Requirements
UVa - Orientation Seminar: **03/06/2008**
UVa - Working Safely with Animals Training: **03/06/2008**
UVa - Animal Facility Rules and Procedures Training: **05/15/2012**
LATA - Mouse Training: **01/22/2008**
LATA - Anesthesia and Analgesia of Rodents: **01/22/2008**