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Research article

Preparing Burn Mouse Model Infected with *Pseudomonas aeruginosa*: a Histopathological Study

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ABSTRACT

The infection of burn injuries by *Pseudomonas aeruginosa* represent a biggest challenges facing doctors. Therefore, treating infected burns area requires finding an animal model that helps standardize the treatment protocols used to treat infected burns, as well as to test appropriate medications. The current study aims to prepare a model of burnt ears as well as those infected with *P. aeruginosa*. In the present study, the posterior portion of mice back was burned using an iron bar heated with boiling water (100 ºC). After one hour, the burn area was contaminated with a standard dose of *P. aeruginosa* (50 µl of 10^8 c.f.u./ml). The changes that occur in burned skin and bacteria contamination were observed with the neck eye, in addition to collect skin pieces (after 48 h) from infected area and put them in 10 % of formalin for histological examination. The results of the histological examination showed damage to the tissues that were burned and that were exposed to the standard dose of *P. aeruginosa*, where the destruction of epithelial layer and damage to the dermis layer was observed, in addition to the appearance of edema, as well as the infiltration of leukocytes. As for tissues taken from mice that were subjected to burning (posterior portion) without contamination with bacteria, minor changes were observed and they quickly returned to their normal status. It can be concluded that the method used was effective in preparing a model of burned mice infected with bacteria, and this will help in conducting experiments to standardize the protocol of treatments.

Keywords: Burn mouse model, Histopathology, Leukocytes, *Pseudomonas aeruginosa*, second-degree burn.

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1. INTRODUCTION

A burn is a type of injury caused by the application of heat, electricity, chemicals, or irradiation to the body. It can result in partial or full-thickness damage to the skin. Burns are classified into thermal, electrical, and chemical categories. They are among the most devastating injuries and a major global public health crisis. Burns can cause various symptoms such as erythema, blisters, and pain. Traditional treatments for burns include topical treatments, special diets, and in some cases, phlebotomy. Burns have significant medico-legal importance and can be considered the most common cause of unnatural death in certain regions. Good early management of burns is crucial for proper resuscitation, but burns are often mismanaged [1-3]. Burn infections have several risk factors. Poor hand hygiene and lack of adherence to wearing Personal Protective Equipment (PPE) and contact isolation precautions were identified as significant contributing factors to acquiring infections [4]. Burn patients are at high risk of infections due to severe impairment of immunity and loss of skin barrier function [5]. The history of antibiotic usage, length of *intensive care unit* stay, mechanical ventilation, and catheter usage were found to be important risk factors for infections associated with antibiotic-resistant Gram-negative bacilli [6]. Multiple factors increase bu-

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burn patients' risk of invasive infection and sepsis, including underlying factors and co-morbidities, the percent total body surface area of the burn, delays in burn wound excision, and microbial virulence/bacterial count [7]. Risk factors associated with time to first healthcare-associated infection (HAI) in burn patients include burn size (TBSA > 20%), burn mechanism (flames and scalds), central venous catheter use, and mestizo race [8].

Burn infections are commonly caused by a variety of bacteria. *Staphylococcus aureus* is a predominant bacterium responsible for burn infections, found in multiple studies [9]. *Pseudomonas aeruginosa* is another common bacterium associated with burn infections [10]. *Acinetobacter baumannii* is also identified as a significant pathogen in burn wound infections [11]. Other bacteria that have been isolated in burn infections include *Klebsiella pneumoniae, Escherichia coli, Proteus mirabilis, Citrobacter freundii, Enterobacter* spp., and *S. epidermidis* [12]. These bacteria exhibit varying levels of resistance to antibiotics, including methicillin-resistant Staphylococcus aureus (MRSA) and extended-spectrum β-lactamase (ESBL) producers. It is important to note that the specific bacteria responsible for burn infections may vary depending on the geographical location and duration of hospital stay [13].

Animal models commonly used for burn studies include small animals such as rodents and large animals such as pigs and sheep. Small animal models, particularly rodents, are more cost-effective and can be used to answer specific questions related to burn injuries [14]. They have been modified to be appropriate for studying burns and have been used extensively in burn research [15]. Large animal models, such as pigs and sheep, have anatomical similarities to humans, making them valuable for studying burn healing, scarring, inhalation injury, and sepsis [16]. However, these models are more expensive and demanding in terms of labor and resources [17]. In addition to these models, some invertebrate models have been developed to study burn trauma and wound infection, providing an alternative to traditional vertebrate models [18]. These models offer the potential for high throughput screening and genetic studies. In the present study, the mice burn model post-exposure to the infection dose of *P. aeruginosa*, and the histological changes in the skin tissue of the mouse were examined.

2. MATERIALS AND METHODS

2.1. Clinical isolates

A *P. aeruginosa* clinical isolate that was previously isolated from infected burn wounds. The isolate was procured from the Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq. Bacterial isolate was preserved by culturing onto nutrient agar slant and incubated at 37ºC for 18 h, and then the slants were kept at 4 ºC for a month.

2.2. Mouse

BALB/c mice 6-8 weeks old, weighing 20-25 gm procured from central animal house, AL-Nahrain University, Baghdad, Iraq. Animals were kept in clean polypropylene cages and fed on the standard antibiotic-free diet. The mice that were used in the current study were male.

2.3. Animal model

In the current study, the standard method of Tavares Pereira Ddos et al. (2012) with little modification to be in line with the aims of the present study was followed to obtain a second-degree thermal burns mice model to evaluate the healing action of therapeutic agents of topical use [19]. Thermal injuries were made with a solid aluminum bar 10 mm in diameter. The bar was previously heated in boiling water. The bar was maintained in contact with the animal skin on the last third of the middle mouse's back for 15 sec. The pressure exerted on the animal skin corresponded to the mass of 100 gm of aluminum bar used in the burn induction. Immediately after the procedure, the mouse was put in a cool place to relax. 50 µl of 10^8 c.f.u/ml of *P. aeruginosa* (washed three times with sterile normal saline) were applied onto the burn region post 1 h of skin burn. The burn area that was contaminated with bacteria was covered by sterile surgical tape. The infected mice should be kept in very clean and sterile cages and fed sterile and antibiotic-free food and water. The mice were scarified and dissected in 3rd days post inoculating with bacteria. The burn and infected area was removed and put in 10 % formalin. The burnt tissue pieces contaminated with bacteria were examined histologically to notice the most important tissue changes that occurred as a result of the burn, as well as, as a result of the effect of the bacterial infection. The results were compared with the skin pieces of healthy mice.

2.4. Histopathological examinations of skin

The standard method of Zgair and Chhibber was followed to prepare formalin-fixed and paraffin-embedded skin sections. The skin pieces of test and control groups of mice were prepared after the animals were killed. Sections of the skin were stained with haematoxylin and eosin [20].

3. RESULTS

3.1. Preparing the burn-mouse model

The burn wound was made in an experimental animal with a special heated bar (cylinder bar, diameter of bar 0.9 cm). The burn areas were contaminated with overnight growth of *P. aeruginosa*. The infected burn wound was developed after 2 days post inoculation with overnight growth of infection dose of *P. aeruginosa* (50µl of 10^8 c.f.u./ml). Fig 1 shows the mouse post two days of burn and contaminated with *P. aeruginosa*. It was seen clearly the infected area and it can be seen the pus overflow from the wound. In this figure also it can be seen the burn that was from II degree, as the completely the skin was destroyed and removed. In this type of burn the skin is only affected. The edema intensity was mild, with no bubbles and the formation of a thick and dry crust from the 2nd day. In the control group, the burn area was red only, due to the removal of the skin layer due to the burn by the heated bar, in addition to the inflammation (redness) occurring as a result of exposure to high temperature, and pus was not seen in the burn area, due to the absence of bacterial infection.

3.2. Histopathological study

The histological examination of the posterior portion of the mouse's back post thermal exposure with a hot bar and after 2 h of contamination with *P. aeruginosa* (50µl of 10^8 c.f.u./ml). The sections made post 48 h of time of contamination when the burn wound got the feature of contaminated wound (pus appear). The sections showed the destruction of the upper layer of skin (epithelial layer) and the destruction reached the mid layer of skin. It can be seen the heavy infiltration of leukocytes confirms the infection with bacteria (*P. aeruginosa*) (Fig 2 c and d). This matched what was seen with the naked eye in terms of noticing ulceration of the burn area and the appearance of pus in the posterior portion of the mouse's back post-thermal exposure with a hot bar and after 2 h of contamination with *P. aeruginosa*.

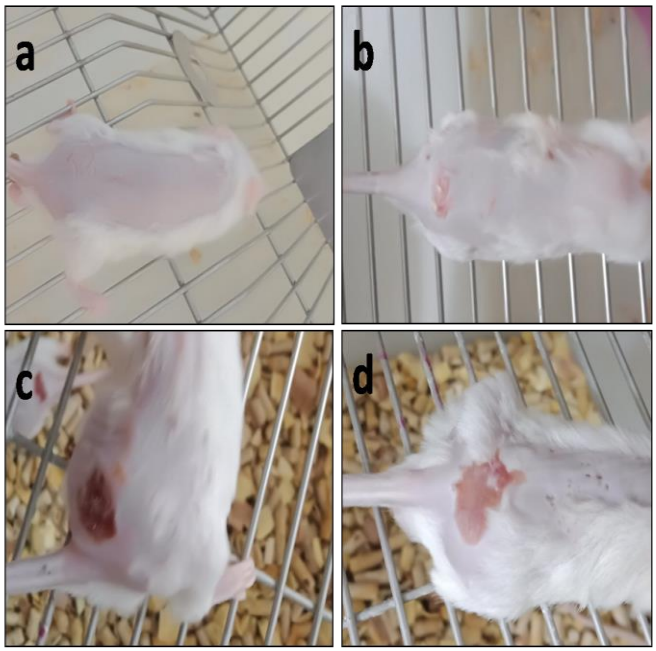

Fig 1. Animal (mouse) burn model. a, The posterior portion of the mouse's back after hair removal. b, The posterior portion of the mouse's back after hair removal and exposure to burning by a hot bar. It clearly shows the removal of the skin due to the burn. c, The posterior portion of the mouse's back two days after it was burned by a hot bar and exposed to the infectious dose of *P. aeruginosa*, it was seen the pus covered the burn area. d, The posterior portion of the mouse's back and two days after it was burned by a hot bar and exposed to sterile normal saline, can be seen only the redness of the burn area without any indicator of infection.

The result of the current experiment was compared with sections of the posterior portion of the mouse's back not exposed to any thermal exposure (Fig 2 a and b). In the sections of control (normal skin) can be seen the normal structure of the skin (all layers) and the hair follicle appeared close to the epithelial layer of the skin. That proved the success of the experiment in terms of getting the burn animal model. This mode will be used in further experiments to identify the possibility of using different substances in treating the infected burn area with *P. aeruginosa*.

4. DISCUSSION

Burn injuries can be classified into different types based on the cause and severity. The types of burn injuries include thermal burns, which are caused by hot liquids, hot solids, or flames [21]. Electrical burns occur due to contact with electricity [22]. Chemical burns result from contact with chemicals. Radiation burns are caused by exposure to radiation or radioactivity [23]. Additionally, burns can be classified based on the depth of the injury, such as superficial, superficial partial-thickness, deep partial-thickness, and full-thickness burns [23]. It is important to note that burns can have local effects on the skin as well as systemic consequences, leading to severe and prolonged inflammatory responses [23]. There are several animal models were used to study the effect of burn on the host and the effect of exposing the burn area to infected bacteria, especially those resistant to different kinds of antibiotics that make the treating of burn infected area very difficult and considered a big challenge to the physicians [14].

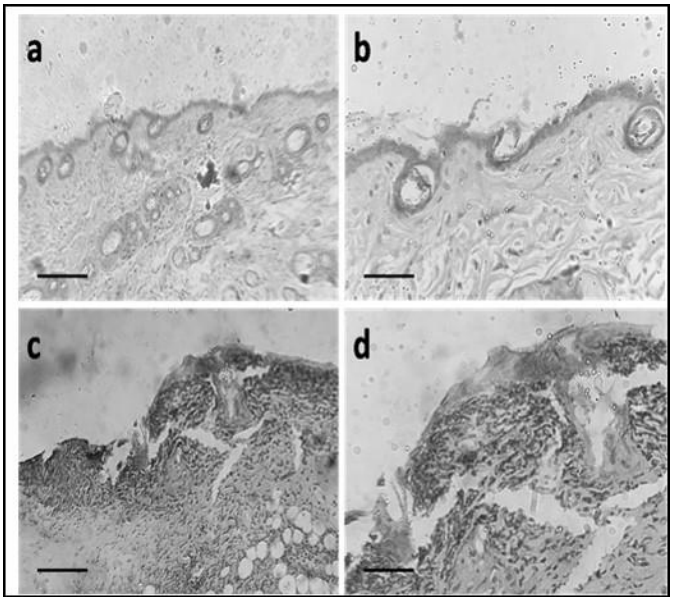

Fig 2. Hematoxylin and eosin staining of mice skin. a, section of normal mice skin showing the normal structure of the skin and the epithelial layer and dermal layer were normal, no heavy infiltration of leukocytes, and the follicle of hair appeared near the surface layer (bar 100 µm). b, High power of normal mouse skin (bar 65 µm). c, Section in mouse skin post thermal exposing with hot bar and contaminated with 50µl of 10^8 c.f.u./ml of *P. aeruginosa*, the result harvested post 48 h of bacterial contamination, the upper layer of skin destroyed and the high leukocyte infiltration (bar 120 µm). d, high power of mouse skin thermal exposing with hot bar and contaminated with 10^6 C.F.U of *P. aeruginosa* the result harvested post 48 h of bacterial contamination, showing the high number of leukocyte infiltration with destroyed of an upper layer of skin (bar 75 µm).

In the current study, mice were used as a model to study the effect of second-degree burns infected by *P. aeruginosa*. The mouse's skin was burned using an iron rod heated with boiling water to produce a second-degree skin burn. The burned area was then inoculated with a standard dose of *P. aeruginosa*. Two days later, the results showed that the skin of the proposed infected mouse with the bacteria had developed a typical burn model attributed to the inflammation phenomenon in addition to ulcerations resulting from the infiltration of leukocytes into the burned-infected area. The development of a model of a second-degree burn wound has a great medical benefit, as it provides an opportunity to test appropriate treatment mechanisms and the possibility of using new methods or the possibility of standardizing methods and medications used in treating burn infections.

The mouse skin contains the major layers of human skin (epidermis, dermis), and there are significant histological and physiological differences of these skin layers to that of humans. For instance, mice have a thinner epidermis and dermis compared to humans [24], and the interphase of the human epidermis and dermis is highly undulated whereas in the mouse it is flat [24]. Also, mouse skin dorsum is covered with dense hair that undergoes a defined cycle of hair growth that is significantly different from human hair. For example, the mouse hair cycle is usually three weeks, whereas human hair cycles can last several years [24].

Additionally, mouse skin is unique in having a distinct panniculus carnosus (a thin skeletal muscle layer found only at the platysma of the neck in humans) [24]. Thus, these are important considerations one should factor in when assessing the translational accuracy of utilizing mice in wound healing studies.

Hiyama *et al.* (2013) prepared a burn mouse model by involving a small (6-8 weeks old) healthy mouse. Initially, they anesthetized mice intraperitoneally with injections of Ketamine and Xylazine. They also gave the mice 1 ml of saline subcutaneously along the spine to cushion the spinal cord from any injury. Following this, the hair on the dorsum is shaved off to ensure even burn wounding. The dorsum is an ideal choice because it is difficult for the animal to reach and as such prevents further injuries to the wound area. The exposed area of the mouse from the template is then immersed in a 100°C water bath for 8 seconds to inflict a full-thickness burn [25]. The temperature (60-100°C) and exposure time (8-12 seconds) vary from study to study; Younan *et al.* (2010) exposed the mouse to 54 ^{0}C for 25 seconds [26].

5. CONCLUSION

It can be concluded from the current study that the model that was prepared was very suitable for conducting future experiments, as the experiments showed the appearance of all the characteristics of second-degree burns in the mouse model prepared in this experiment, where suppuration and destruction of the epithelial cell layer appeared, in addition to the occurrence of filtration of white blood cells. All these features appear only post-bacterial infection.

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Conflict of interest

The authors declare that they have no conflict of interests.

Ethical Approval

This review was approved by the University of Baghdad, Baghdad, Iraq (No 440a, 2023).

Author contributions

Jenan A.Ghafil: Investigation; Methodology; Project administration; Resources; Supervision; Validation; Roles/Writing - original draft; and Writing - review & editing.
May T. Fliyh: Conceptualization, Data curation; and Formal analysis.
Lubna A Abd Al-Mutalib: Roles/Writing - original draft; Visualization and Writing - review & editing.

6. REFERENCES

[1] **Aliasl J, Khoshzaban F.** (2013) Traditional Herbal Remedies for Burn Wound Healing in Canon of Avicenna. *Jundishapur J Nat Pharm Prod* **8**:192–6. Epub 2013 Nov 1. PMCID: PMC3941892.

[2] **Jones SM, Gilbert PM.** (2002) Immediate Care of the Burned Patient. *Surgery (Oxford)* **6**:126-130. doi: 10.1383/SURG.20.6.126.14632

[3] **Hettiaratchy S, Dziewulski P.** (2004) ABC of burns. Introduction. *BMJ* **328**:1366-8. doi: 10.1136/bmj.328.7452.1366. PMID: 15178618; PMCID: PMC420296.

[4] **Al-Benna S.** Protective Measures For Burn Care Professionals During The Coronavirus Disease 2019 Pandemic: Systematic Review. *Ann Burns Fire Disasters* **33**:182-190. PMID: 33304207; PMCID: PMC7680203.

[5] **Corcione S, Pensa A, Castiglione A, Lupia T, Bortolaso B, et al.** (2021). Epidemiology, prevalence and risk factors for infections in burn patients: results from a regional burn center's analysis. *J Chemother* **33**:62-66. doi: 10.1080/1120009X.2020.1780776. Epub 2020 Jun 26. PMID: 32588768.

[6] **Varshochi M, Hasani A, Pour Shahverdi P, Ravanbakhsh Ghavghani F, Matin S.** (2020) Risk Factors for the Antibiotic Resistant Gram-Negative Bacilli Associated Infections in Burn Patients and the In-Vitro Susceptibility of Colistin. *Arch Clin Infect Dis* **15**:e91174. https://doi.org/10.5812/archcid.91174.

[7] **D'Abbondanza JA, Shahrokhi S.** (2021) Burn Infection and Burn Sepsis. *Surg Infect (Larchmt)* **22**:58-64. doi: 10.1089/sur.2020.102. Epub 2020 May 4. PMID: 32364824.

[8] **Escandón-Vargas K, Tangua AR, Medina P, Zorrilla-Vaca A, Briceño E, et al.** (2020) Healthcare-associated infections in burn patients: Timeline and risk factors. *Burns* **46**:1775-1786. doi: 10.1016/j.burns.2020.04.031. Epub 2020 May 16. PMID: 32593482.

[9] **Ruegsegger L, Xiao J, Naziripour A, Kanumuambidi T, Brown D, et al.** (2022) Multidrug-Resistant Gram-Negative Bacteria in Burn Patients. *Antimicrob Agents Chemother* **66**:e0068822. doi: 10.1128/aac.00688-22. Epub 2022 Sep 6. PMID: 36066237; PMCID: PMC9487463.

[10] **Hashemzadeh M, Heydari R, Asareh Zadegan Dezfuli A, Saki M, Meghdadi H, Bakhtiyariniya P.** (2022) Occurrence of Multiple-Drug Resistance Bacteria and Their Antimicrobial Resistance Patterns in Burn Infections from Southwest of Iran. *J Burn Care Res* **43**:423-431. doi: 10.1093/jbcr/irab097. PMID: 34236077.

[11] **Otta S, Dash JK, Swain B.** (2015) Aerobic bacteriology of burn wound infections, *CHRISMED J Health Res* **2**:337. doi: 10.4103/2348-3334.165739.

[12] **Shafaei K, Nahaei MR, Kalhory S, Norani Z, Hosseini M.** (2014) Determination of prevalent bacteria in burn infections compared to patients' environmental bacteria in Burn Ward of Shahid Zarreh Hospital in Sarie, Iran. *Med J Tabriz Univ Med Sci Health Serv* **36**:62.

[13] **Yoon J.** (2016) Bacterial strains and antimicrobial resistance of wound infection in burn patients. *Korean J Fam Pract* **6**:179-84. doi: 10.21215/KJFP.2016.6.3.179.

[14] **Burmeister DM, Supp DM, Clark RA, Tredget EE, Powell HM, et al.** (2021) Advantages and Disadvantages of Using Small and Large Animals in Burn Research: Proceedings of the 2021 Research Special Interest Group. *J Burn Care Res* **43**:1032-1041. doi: 10.1093/jbcr/irac091. PMID: 35778269.

[15] **Grantham LE, Jockheck-Clark AR, Scott LL.** (2022) 811 Statistical Analyses of Multiple Burn Wounds within an Animal. *J Burn Care Res* **43**(Supplement_1): S211-S212. doi: 10.1093/jbcr/irac012.359.

[16] **Hao D, Nourbakhsh M.** (2021) Recent Advances in Experimental Burn Models. *Biology (Basel)* **10**:526. doi: 10.3390/biology10060526. PMID: 34204763; PMCID: PMC8231482.

[17] **Satheesh A, Padukana A, Varghese R, Radhakrishnan A, Dinesh PT, Chandy G.** (2022) Burn inducing device with temperature control to generate experimental cutaneous burn wounds in animal models. doi: 10.51966/jvas.2022.53.1.18-21.

[18] **Maslova E, Shi Y, Sjöberg F, Azevedo HS, Wareham DW, McCarthy RR.** (2020) An Invertebrate Burn Wound Model That Recapitulates the Hallmarks of Burn Trauma and Infection Seen in Mammalian Models. *Front Microbiol* **11**:998. doi: 10.3389/fmicb.2020.00998. PMID: 32582051; PMCID: PMC7283582.

[19] **Tavares Pereira Ddos S, Lima-Ribeiro MH, de Pontes-Filho NT, Carneiro-Leão AM, Correia MT.** (2012) Development of animal model for studying deep second-degree thermal burns. *J Biomed Biotechnol* **2012**:460841. doi: 10.1155/2012/460841. Epub 2012 Mar 29. PMID: 22736951; PMCID: PMC3379528.

[20] **Zgair AK, Chhibber S.** (2010) Immunological and pathological aspects of respiratory tract infection with *Stenotrophomonas maltophilia* in BALB/c mice. *J Microbiol Biotechnol* **20**:1585-91. doi: 10.4014/jmb.1003.03013. PMID: 21124066.

[21] **Jeschke MG, van Baar ME, Choudhry MA, Chung KK, Gibran NS, Logsetty S.** (2020) Burn injury. *Nat Rev Dis Primers* **6**(11): 1-25. https://doi.org/10.1038/s41572-020-0145-5.

[22] **Guoping C, Minlie Y, Shun Y, Hongbo Q, Qingguo Z, et al.** (2014) The middle-forearm flap based on perforator of ulnar artery for electrical burn wound on the wrist. *Zhonghua Zheng Xing Wai Ke Za Zhi* **30**:346-8. Chinese. PMID: 25522485.

[23] **Burgess M, Valdera F, Varon D, Kankuri E, Nuutila K.** (2022) The Immune and Regenerative Response to Burn Injury. *Cells* **11**:3073.

doi: 10.3390/cells11193073. PMID: 36231034; PMCID: PMC9563909.

[24] **Abdullahi A, Amini-Nik S, Jeschke MG.** (2014) Animal models in burn research. *Cell Mol Life Sci* **71**:3241-55. doi: 10.1007/s00018-014-1612-5. Epub 2014 Apr 9. PMID: 24714880; PMCID: PMC4134422.

[25] **Hiyama Y, Marshall AH, Kraft R, Qa'aty N, Arno A, et al.** (2013) Effects of metformin on burn-induced hepatic endoplasmic reticulum stress in male rats. *Mol Med* **19**:1-6. doi: 10.2119/molmed.2012.00330. PMID: 23348514; PMCID: PMC3592932.

[26] **Younan G, Suber F, Xing W, Shi T, Kunori Y, et al.** (2010) The inflammatory response after an epidermal burn depends on the activities of mouse mast cell proteases 4 and 5. *J Immunol* **185**:7681-90. doi: 10.4049/jimmunol.1002803. Epub 2010 Nov 12. PMID: 21076070; PMCID: PMC3058366.

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