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14. ABSTRACT The focus of this grant is to investigate immunoprofiles for serum antibodies to aberrant glycans in human and animal models of mesothelioma. This is accomplished using a one of a kind printed glycan array which is at NYU School of Medicine (NYUSoM). It is hoped that these experiments will allow us to diagnose and prognosticate mesothelioma more accurately in the future. We have been severely limited by our ability to start the human mesothelioma glycoprofiles as well as the animal profiles due to delivery and set up times for our one of a kind glycomics laboratory at NYUSoM. We summarize the situation in the progress report with the good news that we will be moving onwards in June with these studies.					
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INTRODUCTION

This project is funded in order to investigate immunoprofiles of serum anti-glycan antibodies recognizing Mesothelioma-derived aberrant glycans in human subjects and in animal models of Mesothelioma. This is accomplished using a one of a kind printed glycan array which has been developed by us at the New York University School of Medicine (NYU SoM) and has now been expanded by addition of 177 novel glycan probes, many of which are Mesothelioma-specific. It is expected that the results of these experiments will allow us to diagnose and prognosticate Mesothelioma earlier during its development. Results of our experiments using rat model of human Mesothelioma should also provide leads into the immuno-preventive and immunotherapeutic approaches to treatments of the military personnel of high-risk for this malignancy due to their potential long-term exposure to carcinogenic form of asbestos during their service.

BODY

The first arm of our study is carried out as scheduled: asbestos as carcinogen, silica dioxide as a non-carcinogen control for asbestos, and saline as a control for a process of injection have been administered as peritoneal injections and all animals have been bled and weighted monthly. We have collected two "baseline bleeding and weight" measurements prior to all injections. We are delighted to report that we have completed the necropsy part of the experiment. To summarize we have had 72 animals under surveillance for one year. Thirty two animals received intraperitoneal asbestos; 32 others received silica dioxide; and 8 received saline. On October 23, 2012 we completed all of the necropsies for animals surviving 400 days from the time of acclimation and injection. The weights of the three groups are seen in Figure 1.

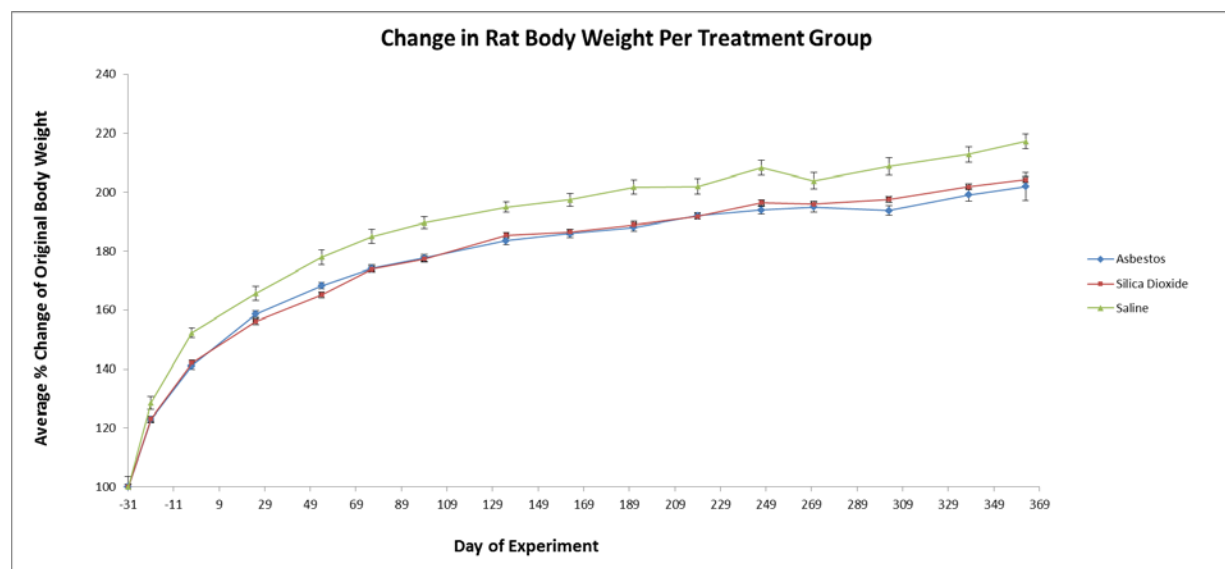


Figure 1: Animal weights over one year

Longitudinal blood sampling was performed fourteen (14) times over the course of the experiment with all blood process for serum and frozen away.

As seen in Table 1, 2/3 of the animals that received the asbestos developed tumors.

Table 1: MPM tumor formation

Treatment	# of Rats	With Tumors	With Minimal Burden of Meso	Normal	% Meso
Asbestos	32	21	4	7	65.625
Silica Dioxide	32	1	0	31	
Saline	8	0	0	8	

At the time of necropsy, all tumors were harvested, cryopreserved in liquid nitrogen along with matching normal liver. All animals had blood harvesting for printed glycan array investigations. Any ascites that was present was also preserved and frozen after processing for cells and fluid.

The final necropsy on these animals was October 23 2012. **Eight days later the entire vivarium at NYU Langone Medical Center was completely destroyed by Hurricane Sandy with the loss of thousands of animals. THE EXPERIMENT DESCRIBED ABOVE WAS COMPLETELY FINISHED AND HARVESTED EIGHT DAYS BEFORE THIS EVENT.** Since that time there have been no vivarium facilities at NYU. Moreover, due to Sandy, we were unable to do any glyco profiling until recently. Our plans are now to perform profiling on our new PGA-400 of these over 1000 blood specimens as well as selective profiling of the ascites.

Printed Glycan Array

In preparation for the immunoprofiling of serum anti-glycan antibodies (AGA) in populations of asbestos-exposed individuals and patients with Malignant Pleural Mesothelioma, and in the animals in all arms of our study employing rat model of human Mesothelioma described in our original study plan, we have developed an expanded version of our glycochip, NYU PGA-400. Our next generation of printed glycan array (PGA) includes now 377 synthetic glycans of pharmacological purity. Majority of novel glycan probes has been designed based on our experimental results obtained with the previously utilized PGA-200. Our current glycan library includes expanded categories of N-glycans such as fucosylated, sialylated and sulfated complex lactosamines, extended and modified blood group A, B, I and P glycans, extended and modified Lewis (Le)a, Leb, Lec, Lex and Ley glycans, as well as multiple tumor-associated glycolipid glycans, sialylated, sulfated and modified O-glycosylation core structures, synthesized on different amino- spacers that include peptide mimics and extended hydrophobic units. These glycans have been synthesized in the laboratory of Prof. Nicolai V. Bovin (Russian Academy of Sciences, Moscow, Russia). NYU PGA-400 glycochips are printed at 50 and 20 μ M concentrations, at eight replicates of each glycan at both concentrations. These glycochips are reproduced and quality-tested by a set of procedures that have been standardized and

optimized antibodies binding to printed glycans are performed as described in Huflejt et al. (2009) and Vuskovic et al. (2011) for pre-clinical diagnostic research applications.

KEY RESEARCH ACCOMPLISHMENTS

1. We have completed the longitudinal animal experiment with successful blood draws.
2. We established a 65% tumor rate with this model.
3. We have a valuable one of a kind resource of asbestos induced mesotheliomas.
4. We have established the new glyco-laboratory with the dedicated print-room which now allows printing of large batches of glycochips of an enhanced quality. Now that we are over the aftermath of Sandy, we can begin printing.

REPORTABLE OUTCOMES

None

CONCLUSIONS

1. Our new glyco-lab with its dedicated, nearly particle-free print-room allows us now to print glycochips of much improved quality, and with the higher efficiency. We now have all the materials from the experiment and we will begin antibody analyses

REFERENCES

Huflejt ME, Vuskovic MI, Vasiliu D, Xu H, Obukhova P, Shilova N, Tuzikov et al.

[Anticarbhydrate](#)

[antibodies of normal sera: findings, surprises and challenges.](#) *Mol Immunol*

Vuskovic MI, Xu H, Bovin NV, Pass HI, Huflejt ME.

.
2009; 46: 3037-3049. PMID: 19608278.

[Processing and analysis of serum antibody](#)

[binding signals from Printed Glycan Arrays for diagnostic and prognostic applications.](#) *Int J*

Bioinf Res App. 2011; 7: 402-426. PMID: 2211253.