

19/10

BioMI 290

Case Study 2 – Flu

+1 1) The “severe acute respiratory syndrome” could possibly be caused by Gram (-) or Gram (+) bacteria, a virus, or by fungi. Any of these could get into the respiratory tract and cause the infection.

+2 2) Gram staining is a differential staining technique in which uses a two types of dye to differentiate Gram – and + type bacteria. A crystal violet-iodine complex is used to detect Gram + bacteria as it is taken up by the thick cell wall making them a purple color. Gram – bacteria do not have a thick wall and have an outer membrane and safrinin is used to dye them pink. The Gram staining results were pretty vague. Of the 4 patients tested, Patients 2, 3, and 4 seemed to either have no abnormal bacteria present, or they were gram -, while Patient 1 may have had either gram + or gram – bacteria or nothing at all, but it is hard to actually tell since it is so dark. None of the bacteria observed look decisively like the any of the results for these patients and from the results, the apparently Gram + cocci can be ruled out and it is possible that none of these bacteria is responsible since no bacteria really appear to be present at all.

+2 3) Fluorescent antibody (DFA) technique uses the reflection of certain wavelengths of light to detect a particular antigen against a dark background. In DFA, Fluorescent chemicals are attached to a constant region of an ^{antibody} antigen and this generates a specific protein tag which is used to identify that antigen. After time is given to bind and the fluorescent chemical is rinsed off, the specific antigen for that chemical should fluoresce and be seen if it is present in the sample. Of all the patients tested for several viruses using this technique, the only positive results were in patients 2 and 3, who showed the presence of Human Respiratory Syncytial Virus. Results of tests for Rhinovirus, influenza, and human adenovirus all came up negative. These results seem to suggest Human RSV as the cause, or at least that a virus is present in these patients. The RSV, although apparently present, is still not in all of the patients, so it is likely not the only virus present.

+2 4) Before the electron micrographs are taken, separate cultures of cells from other mammalian lines must be grown and then inoculated with sputum samples from the patients. This is because there may be only a few pathogens in a particular sample which must be given a chance to grow efficiently in the new culture. If the virus from the patients is cultured and then put into other cell lines, it can be studied after the first sign of any cell damage. By using this method, cytopathic effects can be identified on a daily basis and then observed using the electron microscope. Using a bombardment of electrons from the microscope, an image is developed of whatever is in the sample and this can be used identify any pathogens. In this case, a few viruses are detected in the EM results. As in the DFA analysis, it was found that patients 2 and three had some virus similar to RSV or possibly Influenza. This is evident from the growth of a virus like this in Culture A. Since the other two patients did not lead to these results this does not look like it is the major cause of the disease. Culture C is more telling in that each patient

ended up transferring a similar pathogen to it. From observing EM results it appears that the virus existing in all patients is a Human Coronavirus the size and shape of the EM results from the experiment look very similar to coronavirus images in the viral library.

+3 5) After analysis of the results, it appears that a human coronavirus is the cause of the flu being studied. Gram stain results basically ruled out a bacterial influence since no bacteria are evident in any patient. The DFA results do show that a virus is present in at least some of the people suffering from the disease but it is not likely RSV which shows up in only a few patients. The results are still vague and the EM test is needed to see if there is a specific Virus that seems to be common to all four studied patients. After observing a virus that looks very much like a coronavirus (in size, shape, and apparent consistency) coming from all four patients, it seems that the only pathogen possibly responsible is this type of virus. All cells in culture C have the virus, but its absence in the other two cultures may just be because these cells are not as susceptible. Since it was not studied in the DFA test, the coronavirus would not have been identified there, but further testing using DFA may help to further identify coronavirus as common among all patients. The apparent RSV does show up in the same two patients when EM is used but it is not consistent among all patients so it can't be the cause of disease. A human coronavirus may not have been initially tested for or expected because it usually mild in humans and would not be expected to lead to such a dangerous outbreak. The fact that coronavirus has not been a problem like this makes it surprising, but all results point to this kind of virus as the only potential cause of the disease.