Empirical evidence that the blood on the Shroud of Turin is of human origin: Is the current data sufficient?

Abstract

Previous studies have established that the blood areas on the Shroud of Turin consist of real blood and are not composed of artistic pigments. It is commonly reported that the blood is of human origin, but just how substantial is the evidence to support this conclusion? Here, the empirical evidence for the human origin of the Turin Shroud bloodstains is evaluated. As discussed, the majority of serological data supports the idea that the blood is indeed primate, but a confident conclusion that the blood is indisputably human is somewhat confounded by the cross-reactivity of many "human-specific" reagents with blood of other species. The potential usefulness of additional testing methods, many developed since the original studies were performed approximately thirty years ago, is also discussed.

Blood and blood components

The human body contains approximately five liters of blood, which is continuously pumped throughout the circulatory system during life. Blood consists of two basic components: the fluid portion (serum) and the cellular portion. Numerous types of proteins exist within the serum fraction, the most prevalent being albumin, important for maintaining the protein concentration (osmolality) of the blood, and immunoglobulin, or antibody, which is a product of the immune system (Figure 1). Together, albumin and immunoglobulin account for approximately 80% of all proteins present in blood serum (1,2).

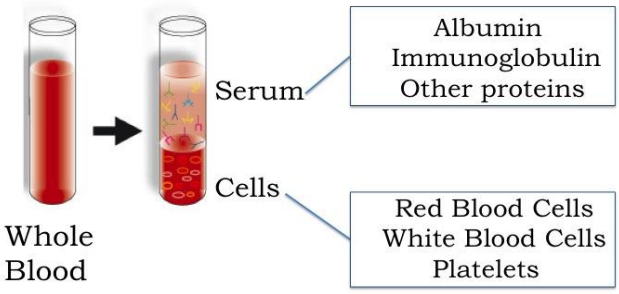

Figure 1. Whole blood consists of serum and cellular fractions. *The serum portion contains albumin, immunoglobulin, and other proteins. The cellular portion consists of red blood cells, white blood cells, and platelets. See text for details.*

The cellular portion of blood is made up of three major blood cell types: red blood cells (erythrocytes) which contain hemoglobin, important for oxygen transport in the body; white blood cells that function in defense against foreign organisms and disease, i.e. the immune system; and platelets, important in clotting (Figure 1). Technically, platelets are not true cells, but rather cell fragments generated from larger cells in the bone marrow (known as megakaryocytes), (1,2). Red blood cells constitute approximately 40% of the blood volume; in a typical microfluid portion of blood, red blood cells outnumber white blood cells by approximately 500 to 1. On average, the human body contains approximately 20-30 trillion red blood cells, having a lifespan (turnover) of between 100-120 days (1,2). Platelets are much greater in number than white blood cells, but because of their relatively small size, account for only a small fraction of the blood volume.

Testing for the presence of blood

The most common chemical tests that are used to determine if a sample is indeed blood involve reaction with the ring structure of hemoglobin, which contains bound iron. A positive test for hemoglobin is indicated by a unique color change, which is easily visualized with the naked eye (2). Additionally, a more detailed technique called spectroscopy may be used, in which a diagnostic machine measures the sample at specific wavelengths of light and generates a corresponding graph. By comparing graph profiles of known substances, one can determine the composition of the sample. Hemoglobin represents a major peak in such patterns (termed the Soret band), and is one of the reference peaks used to confirm that the sample is in fact, blood. Further testing may also be performed to detect other characteristic blood components such as fibrin, fibrinogen, heme breakdown products (including bilirubin), and trace elements.

Multiple chemical studies have clearly demonstrated that blood components are present in the blood areas of the Shroud (3-6). However, such analyses can only be used to verify that blood is present; these types of tests do not distinguish from which species the blood may belong to. For this, additional experimentation using other techniques must be performed, as described below.

Distinguishing human blood from animal blood

In fresh bloodstains, human blood may be distinguished from the blood of a few animal types by simple microscopic examination. Unlike mammalian red blood cells, which lose their nucleus upon maturation, red blood cells from birds, amphibians, and fish maintain a nucleus throughout their lifespan, which is easily seen at low magnification (7). If enough white blood cells are present in the sample, a technique known as karyotyping may be performed (at significantly higher magnification), which examines the number and type of specific pairs of chromosomes that exist in the nucleus (2). Among primates, only humans contain 23 pairs of chromosomes (22 pairs of non-sex chromosomes and 1 pair (XX or XY) of sex chromosomes), for a total of 46. Chimpanzees, gorillas, and orangutans contain an additional pair of non-sex chromosomes (23 pairs plus 1 pair of XX or XY), for a total of 48. The numbers

of chromosomes for other species is quite variable, for example pigs have 38, sheep have 54, dogs have 78, and cows have 60 (8).

In aged bloodstains, such microscopic tests are not practical as cells become dehydrated and rupture within hours of drying. Rather, distinguishing human blood from animal blood in dried samples relies on the detection of specific blood products, by a method known as serological analysis or serology. For these studies specific blood components (usually albumin or immunoglobulin proteins, Figure 1) are detected using antibodies that are created in a species other than human, for example, rabbit or mouse (1,2). Such antibodies are able to specifically recognize regions on human proteins that are distinct from those in the host species in which they are produced. Chicken blood would not react in such tests, because even though albumin (or immunoglobulin) is present, enough difference exists from human albumin (or immunoglobulin) that the antibody fails to recognize it. Similarly, blood from many other common types of animals are distinguished in such tests, including that of cows, horses, goats, sheep, cats, dogs, etc. Quite often a positive reaction in such tests results in the conclusion that human blood is present. However, this is where things get somewhat arduous. Species that are closely related to humans (i.e. apes and monkeys) express albumin (and immunoglobulin) that is similar enough to human protein to react positively in such tests (1,2,9). Typically this is disregarded unless special circumstances warrant that such possibilities be considered (at a crime scene within a zoo, for example, or if someone were known to keep apes or monkeys as pets). In most situations, when it is stated that bloodstains tested positive for human blood, this underlying supposition exists. Strictly speaking, such tests do not distinguish human blood from the blood of other primates (monkeys or apes).

Regarding the Shroud, Heller, Adler and coworkers directly demonstrated such cross-reactivity in their studies by showing that the “human-specific” antibodies used in their experiments also reacted with blood from non-human primates (3-6). Thus, they were only willing to definitively conclude that the blood was (at least) primate blood. In related studies by Baima Bollone and coworkers to characterize bloodstained fibers of the Shroud, cross-reactivity of their “human-specific” reagents was not reported, but certainly the same limitations apply (10-12). Similarly, Garza-Valdez reported reactivity of bloodstained fibers with antibodies directed against adult human hemoglobin, but no details regarding the genuine specificity of such antibodies were available (13, and accompanying note). Given the high sequence homology between hemoglobin proteins expressed in human and non-human primates (14), it is reasonable to expect that such antibodies would cross-react with hemoglobin expressed in similar species.

In the early 1980s, Baima Bollone and colleagues reported that the bloodstains of the Shroud were of the blood type AB (11, 12). The typing studies have been extensively reviewed elsewhere (15) and will not be discussed in detail here, except to say that the ABO antigens are shared between human and certain non-human primates; thus, in of itself, ABO typing cannot specifically distinguish between human blood and blood of other primates, although it may narrow

down the list (15). Several years later, Baima Bollone, et al. continued their serological studies on the Shroud using antibodies as molecular probes for additional blood components found on red blood cells, specifically the M,N, and S antigens. In a brief report, the authors showed that the bloodstains may be further characterized as being MNS positive (16). What is most significant about these studies is that unlike M and N antigens, which are shared between certain primates and humans, the S antigen is exclusive to humans only. No S counterpart exists in other species, including apes or monkeys (17). This point was not emphasized (or mentioned) in the report, as the significance of this relationship among primates was not fully elucidated until several years later (in non-Shroud related studies), (17-19). With the exception of this singular report, all current immunological evidence is unable to effectively distinguish human versus non-human primate blood. To date, this brief study remains the single most definitive piece of serological evidence that directly addresses the human origin of the blood on the Shroud.

Human DNA on the Shroud

In discussions on the human nature of the blood on the Shroud, it is often mentioned that human DNA has been found in the bloodstains. In the 1990s, Garza-Valdes reported in the book "The DNA of God" the cloning of three human gene segments from blood remnants on the Shroud: the betaglobin gene (which encodes a portion of the hemoglobin protein) and the amelogenin-X and amelogenin-Y genes, (specific for proteins encoded on the X and Y sex chromosomes, respectively), (13).

The human genome is estimated to contain approximately 20,000-25,000 protein-encoding genes, a much smaller estimate than originally proposed (20), which is contained within 23 pairs of chromosomes. Cloning in the context of the Garza-Valdes studies means to copy a portion, or segment, of three of these genes from the entire complement of DNA that exists within a human cell. This is analogous to opening a very, very large book and copying several sentences from three separate paragraphs located on three different pages within three entirely different chapters. Just as reproducing a few sentences does not begin to approach recreating the entire book, such experiments do not come remotely close to the creation of a human being, a point that is sometimes misunderstood.

Results from the Garza-Valdes studies indicated that the blood on the Shroud had belonged to a human, and more specifically, a male (13). One issue that is raised in such experiments is that of DNA contamination, i.e. did the DNA that was sequenced truly originate from blood cells present on the cloth; or might there also be contribution from so-called touch DNA: DNA transferred by contact from other persons directly or indirectly through a handled object that came in contact with the cloth. Because the abovementioned genes are not exclusive to blood cells, but are also found in numerous other cell types, including skin, this remains somewhat of an open issue (15).

The human betaglobin gene consists of three coding segments (exons); the specific sequence that was examined in these studies lies within the first coding

segment (exon 1) and is approximately 265 base pairs in length. (A base is one of the four DNA sequence codes, or nucleotides, which are usually indicated as the letter abbreviations A,G,C, or T). By comparing both the nucleotides that are present and the specific order in which they are arranged, one can confidently identify a particular gene; similar to evaluating letters of the alphabet and their particular arrangement into words when studying a written document. For the gene segment that was evaluated, there is an extremely high homology among betaglobin genes expressed in most primates. Humans differ from closely related species (chimpanzee and gorilla) by only a single nucleotide within the entire 265 base pair region (Figure 2); the rest of the gene is identical in all three species (21). Other primates such as orangutan and macaque differ from humans by 5 and 6 nucleotides in this segment, respectively (21),(Figure 2). While a single nucleotide difference may be sufficient to distinguish human DNA from those of related primates, this would certainly fall within the accepted possibility of sequencing error, which can occur during the copying and cyclic amplification of genes in such experiments; it is unknown how many replicate

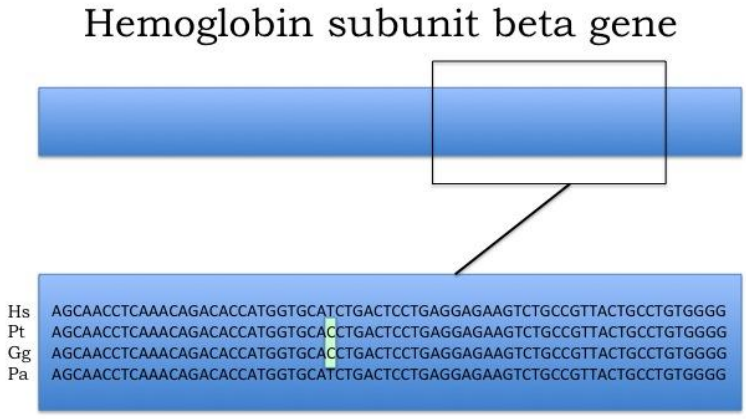

Figure 2. Hemoglobin subunit beta (betaglobin) gene. *A portion of the betaglobin gene sequence is shown. Highlighted is the single nucleotide difference between humans and other related primates. Hs (Homo sapiens = human), Pt (Pan troglodytes = chimpanzee), Gg (Gorilla gorilla = gorilla), and Pa (Pongo abelii = orangutan). The orangutan sequence is the same as human in this region but contains 5 nucleotide differences in other regions of the gene (not shown). Gene alignments were performed using the Ensembl genome browser alignment program (http://useast.ensembl.org/index.html).*

analyses were performed (13). The results would be more decisive if additional betaglobin gene segments containing further nucleotide differences had also been cloned. (No nucleotide sequence data for amelogenin X or amelogenin Y genes was published in these studies), (13). Thus, similar to serological studies, high homology between betaglobin DNA of human and certain related primates

may curtail a more definitive conclusion from these experiments. Regarding these results, Dr. Garza-Valdes himself stated that "*But at this stage, all we could say about the blood was that it was ancient, because of the degree of degradation on the small amount of blood we found on our sample, and that it had come from a human being or high primate*" (13).

Is primate good enough?

Careful consideration of the current data raises several important questions. Are the existing data sufficient to claim beyond a reasonable doubt that the blood is of human origin? Does the unlikeliness of a forger procuring non-human primate blood to use in creation of the Shroud reduce the necessity of auxiliary studies? Importantly, has this area of research advanced significantly in the last thirty or so years such that newer methodology is now available that could confirm and extend previous findings on the characterization of the Shroud bloodstains?

While one may debate about the likelihood of apes or monkeys being readily available to an artist intent on creating a realistic forgery, such discussion is purely anecdotal. Moreover, a committed forger could have used human blood as the medium. At issue here is the empirical evidence for the characterization of the blood that is present on the cloth. Adler has rationally commented on the difficulties a forger would encounter in trying to apply (clotting) blood to various regions on the Shroud (6). The intent of this article is not to suggest that non-human (primate) blood was spread onto the cloth, but rather to objectively examine the strength of the current data in directly demonstrating that the blood is in fact of human origin. To reverse the context, if a sample of (non-human) primate blood were run in parallel in all of the previous experiments, could the sample be readily distinguished by such analysis? As shown in Table 1, while most tests support the conclusion that the blood is (at least) of primate origin, there is only a single (brief) serological study that extends this conclusion to a human origin. (Whether DNA sequencing studies are included as additional evidence for this conclusion is somewhat subjective, and is left at the discretion of the reader; see (15) for discussion).

Additional (Future) Blood Testing

Since many of the original blood characterization studies were performed on the Shroud some thirty years ago, significant advancements have been made in certain techniques that are now available for a more detailed investigation. One of the most important advances in the field of serology is the development of monoclonal antibodies, which were just coming into use in the early 1980s. Unlike previous polyclonal antisera which contain a mixture of different antibodies that are typically reactive with multiple sites on a protein, monoclonal antibodies recognize a specific, single site, greatly increasing their specificity and sensitivity. Importantly, the generation and selection of monoclonals that are species-specific (not cross reactive) is much more efficient than with previous

Table 1. Summary of Blood Testing Studies on the Shroud

Type of Test	Blood component (serum)	Origin
Serological	Albumin[1,2]	Primate
Serological	Immunoglobulin[1,2]	Primate
Serological	Hemoglobin[3]	Primate

Type of Test	Blood component (cells)	Origin
Serological	ABO antigens[2,3]	(Primate)
Serological	MN antigens[2]	Primate
Serological	S antigens[2]	Human
DNA sequencing	Betaglobin[3]	Human?

Table 1. Summary of blood testing studies related to the origin of the blood on the Shroud of Turin. *[1]Studies by Heller and Adler, see References 3-6; [2] Studies by Baima Bollone et al., see References 10-12; [3]Studies by Garza-Valdes, see Reference 13. For ABO testing, results are included in parentheses as certain ABO blood groups may distinguish between humans and some non-human primates. DNA sequencing results are indicated with a question mark because of open issues regarding human-specific target sequences and possible contamination with exogenous DNA. See text for details.*

polyclonal antisera (15). Thus, not only are more antibodies to other blood proteins currently in existence, antibodies that are truly specific for human blood components, which do not cross react with those of even closely related primates, are now available.

General Considerations

In any future testing that may occur, it is important to consider the overall value of such tests in confirming and extending previous studies to characterize the Shroud bloodstains. As the types of tests described below are predominantly destructive in nature, and the amount of samples available for study may be particularly limited, a detailed assessment of their benefit is especially warranted. As previously noted, it would be most informative to evaluate parallel samples taken from several areas for comparison of results (22). Enhanced sensitivity of more modern technology relative to that available in the past may allow experimentation on much smaller amounts of material.

Moreover, consideration of the use of additional methods, particularly those using a sequential approach, would allow multiple sets of information to be obtained from a single sample, conserving significantly the amount of material that may be required. Many previous serological studies relied on microscopic evaluation of labeled antibody binding to samples, which are relatively limited in terms of quantitation, and to some degree, sensitivity. Enzyme-linked immunoassay (ELISA) uses a similar principle in antibody detection, but requires much less material and the degree of antibody binding is not as subjective, as it is mechanically calculated. In addition, a major advantage of this technique is that it can be used in sequential fashion to evaluate multiple components from a single sample. For example, if one wished to assay five different blood components (A, B, C, D, & E), using a conventional approach this would require at least five different samples (Figure 3). In a sequential

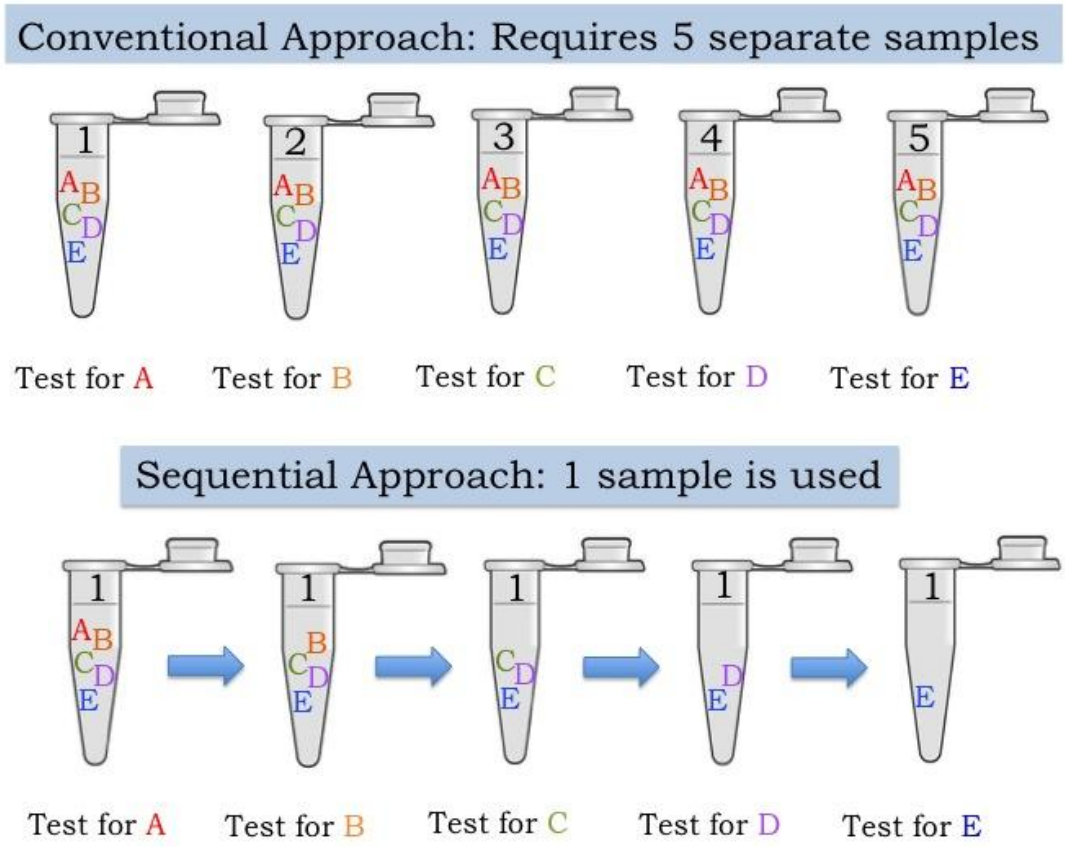

Figure 3. Sequential analysis of the hypothetical blood components A-E. *In the conventional approach, a separate sample is required for the analysis of each component and samples are discarded after use. In the sequential approach, a single sample is first analyzed for A, then remaining material analyzed for B, then for C, etc. This approach is especially useful when the amount of sample is limited.*

approach, a sample is not used a single time and then discarded; rather, component A is analyzed first, then the remaining material is analyzed for B,

followed by C, etc., (Figure 3). Sequential techniques have been used successfully in the analysis of multiple immune receptor proteins and other blood components (23-26). Using a small volume of blood, Osuchowski et al. measured up to fifteen different secreted molecules in five consecutive cycles without inter-assay interference or deterioration of sample (25). The use of such techniques could increase the amount of information that is obtained, while at the same time, reducing the amount of sample that is required. Such methods also make the inclusion of duplicate and triplicate samples within the same experiment much more practical.

Serological testing for human glycophorin A

The single most important test to be done on the Shroud of Turin bloodstains is to unequivocally establish their human origin. In 2010, a monoclonal antibody specific for human glycophorin A became available, allowing rapid and specific identification of human blood for the first time (27). Glycophorin A is membrane protein expressed on the surfaces of red blood cells in humans and other primates (28). The diagnostic and specification studies of this antibody demonstrate no cross-reactivity with non-human primates, including bonobo (chimp), gorilla, orangutan, and baboon. Reactivity is also negative with the blood of unrelated animals such as dogs, cats, horses, chickens and pigs. Data from these experiments could establish conclusively that the blood is of human origin and confirm and extend previous blood characterization studies that were inherently limited by cross-reactivity. Moreover, definitive demonstration of the human nature of the bloodstains by such tests would validate the use of other "anti-human" reagents in the future that show some cross-reactivity with blood components of closely related primates.

Serological and DNA testing for ABO antigens

As current blood typing data on the Shroud relies primarily on forward typing methods (15), much of which was obtained using polyclonal antibodies, it would be informative to perform additional typing studies using monoclonal antibodies, particularly from multiple sites on the cloth (22). It would also be valuable to consider attempting studies at the molecular level, as DNA analysis would circumvent possible objections raised with serological methods (15), and greatly extend these results. The sensitivity capabilities of the polymerase chain reaction (PCR) technique in modern analysis of (fragmented) DNA are such that it might be sufficient to generate meaningful results.

Serological and DNA testing for HLA antigens

Human leukocyte antigens (HLA) are the major histocompatibility complex (MHC) group proteins essential to immune function in humans (1). In the course of organ transplantation, when a person is "typed" to determine if they are a suitable recipient/donor for a particular organ, it is the HLA antigens that are being evaluated. Two sets of HLA molecules exist, class I molecules (HLA-A, HLA-B, and HLA-C) and class II molecules (HLA-DP, HLA-DM, HLA-DOA, HLA-DOB, HLA-DQ, and HLA-DR), which are expressed on many cells throughout the

body, including white blood cells (but not red blood cells), (1,2). HLA antigens are the most polymorphic molecules that exist in humans, meaning that multiple forms of these genes (alleles) are present within the population. For example, more than 2700 different alleles have been reported for the HLA-B molecule (1,29). HLA genes are inherited in a typical Mendelian fashion (one copy from the mother, one copy from the father). Study of HLA expression can provide information regarding ethnicity and the kinship of individuals and populations. Indeed, because of the tremendous diversity that exists among HLA antigens, even a partial HLA profile can yield valuable information about the possible relatedness of two individuals (e.g. the relationship between the Shroud bloodstains and those on the Sudarium; such data is much more definitive than simply having a shared blood type). No information currently exists on expression of HLA molecules related to the Shroud. The feasibility of such studies could be undertaken using a similar approach as that for ABO antigens, e.g. serological and DNA testing.

Serological and DNA testing for other red blood cell antigens

In addition to the ABO antigens, which are the most commonly studied because of their importance in blood transfusion, over 30 other groups of red blood molecules exist, including the previously mentioned MNS antigens. MNS expression was evaluated using polyclonal sera (16); it might be informative to extend these studies through the use of more modern monoclonal antibodies and to consider examination at the gene level.

Expression of the Rh factor, typically listed after an individual's blood type as positive or negative, was not previously evaluated in Shroud bloodstains because it was reportedly too degraded to study at the protein level (15). Numerous anti-Rh monoclonals are now available that might increase the sensitivity of detection in such studies; evaluation of bloodstains at multiple locations might also prove helpful in determining the uniformity of the these findings in multiple areas of the cloth. The Rh gene is located on chromosome 1; if sufficient DNA was intact from this region, this might offer an alternative approach.

Similar to HLA antigens, the Kell blood group antigens, encoded by the KEL gene, are also highly polymorphic. Behind the ABO and Rh blood groups, these are the third most potent in triggering an immune reaction in transfusion reactions that are mismatched (30). As with HLA antigens, identification of which forms of Kell antigens were present might provide important information regarding ethnicity, and also the possible relationship of Shroud and Sudarium bloodstains.

Serological testing for hemoglobin, bilirubin, and Band III proteins

Chemical and spectroscopy studies have established that hemoglobin is present in the blood areas of the Shroud. Although Garza-Valdes reported positive staining of bloodstained fibers with anti-hemoglobin antibodies, no specifics (or data) were published (see 13 and accompanying note). Multiple monoclonals now exist that are specific for hemoglobin that would confirm and extend previous findings. Heller and Adler's conclusions regarding the presence

of bilirubin in bloodstained areas were derived primarily through spectroscopic analyses (3,4,6). Monoclonal antibodies specific for bilirubin are now available which could help verify these results serologically. In addition, more comprehensive chemical analysis of the presence of bilirubin could be performed using of high performance liquid chromatography (HPLC), which requires only a relatively small amount of sample (31). Bilirubin might be of particular interest in that it has been suggested to be an important factor for the unusual red color of the blood on the Shroud (6). In fresh blood, bilirubin content is quantitated by normalizing per unit volume of blood. Bilirubin estimations in aged bloodstains would be more difficult; semi-quantitative analysis might be achieved by normalizing bilirubin content relative to the amount of another blood component, for example, albumin.

Band III is an important structural protein in the red blood cell membrane, to which over 80% of ABO molecules are attached. An individual red blood cell expresses approximately one million Band III molecules, which make up about 25% of its cell surface (32). Evaluation of the expression of Band III could help to provide further evidence that (human) blood exists on the Shroud. Isolation of Band III proteins might also provide a means for the purification of ABO antigens associated with red blood cell membranes (15). Such an approach would effectively nullify the issues of "false positives" with ABO typing, suggested to result from contamination with bacteria and other organisms (15).

Isolation and sequencing of mitochondrial DNA

In contrast to nuclear DNA, which occurs in the form of one to two copies per cell (one copy for sex cells: sperm and egg; two copies for other cell types), most human cells contain approximately 1,000-10,000 copies of mitochondrial DNA per cell; a cell contains a single nucleus, but has numerous mitochondria, necessary for providing energy for various cellular functions. The high copy number of mitochondrial DNA often proves useful under conditions of limiting amounts of sample, such as DNA degradation. Mitochondrial DNA is especially valuable in determining the identity and relatedness of individuals, because unlike nuclear genes in which one copy is inherited from each parent, mitochondrial DNA is received from the mother only (33). The chance of two unrelated individuals having the same mitochondrial DNA is extremely low. The mutation rate of mitochondrial DNA is enhanced relative to nuclear DNA, allowing for a more thorough investigation into ancestry. A comparative analysis of mitochondrial DNA isolated from the bloodstains on the Shroud and those on the Sudarium might offer the most definitive data regarding the relationship of these two artifacts.

Summary

The current paper evaluates the empirical evidence that the blood on the Shroud of Turin is of human origin. While the majority of serological data support the idea that the blood is (at least) primate, only a single (brief) study directly addresses the issue of the human nature of the bloodstains. For most

studies, the shared homology between human and primate blood components limits an indisputable conclusion that the blood is human. Current DNA studies are not particularly helpful due to the extreme similarity of the cloned and sequenced betaglobin gene region in humans and related primates; in addition, the issue of endogenous versus exogenous DNA remains somewhat unresolved. Finally, the usefulness of additional testing methods, many developed since the original studies were performed approximately thirty years ago, in the analysis of the Shroud bloodstains is discussed.

Acknowledgements

Thank you to Barrie Schwortz and Tammy Walden for critical reading of the manuscript. As always, thanks to my wife, Kathy, for everything.

References

1. Deves, P. *Roitt's Essential Immunology*, Wiley-Blackwell, Hoboken, NJ (2006).

2. Rodak, et al. *Hematology: Clinical Principles and Applications*, Saunders Elsevier, St. Louis, MO (2007).

3. Heller, J.H. and A. Adler, "A Chemical Investigation of the Shroud of Turin", *Canadian Forensic Society Scientific Journal* 14: 81-103 (1981).

4. Heller, J.H. *Report on the Shroud of Turin*. Houghton-Mifflin Co., Boston, MA (1983).

5. Jumper, E.J. et al., "A comprehensive examination of the various stains and images on the Shroud of Turin, *ACS Advances in Chemistry, Archaeological Chemistry III* 205: 447-476 (1984).

6. Adler, A. "The Orphaned Manuscript, A gathering of Publications on the Shroud of Turin" *Shroud Spectrum International Special Issue, 1st Edition*, ed. D. Crispino, Effata Editrice, Cantalupa (Torino), Italy (2002).

7. Kingsley, P.D. et al., "Yolk sac-derived primitive erythroblasts enucleate during mammalian embryogenesis", *Blood* 104: 19-25 (2004).

8. Chromosome numbers in different species (1998)
http://www.vivo.colostate.edu/hbooks/genetics/medgen/chromo/species.html

9. Black, C.M. et al. "Human markers for IgG2 and IgG4 appear to be on the same molecule in the chimpanzee", *Immunology* 72: 94-98 (1991).

10. Baima Bollone, P.L., et al., "La Dimonstrazione della presenza di trace di sangue umano sulla Sindone", *Sindon* 30: 5-8 (1981).

11. Baima Bollone, P.L, et al., "Identification of the group of the traces of human blood on the Shroud," *Shroud Spectrum International* 6: 3-6 (1983).

12. Baima Bollone, P.L.. "*The Forensic Characteristics of the Blood Marks" In The Turin Shroud: Past, Present, and Future*, ed. S. Scannerini and P. Savarino [Int.

Scientific Symposium, Torino, Mar. 2-5, 2000], 125-135. Torino, Italy: Effata Editrice (2000).

13. Garza-Valdes, L., *The DNA of God?* Doubleday, New York, USA (1999).
Note: In the course of preparing this manuscript and attempting to contact Dr. Garza-Valdes, it was learned that he passed away in November of 2010. No further information regarding the details of these studies (sequencing data, serological data) have been published.

14. Barnabas, J. et al., "Evolution of hemoglobin in primates and other therian mammals", *Comparative Biochemistry and Physiology, Part B: Comparative Biochemistry* 39: 455-482 (1971).

15. Kearse, K.P., "Blood on the Shroud of Turin: An Immunological Review", http://www.shroud.com/pdfs/kearse.pdf, 2012.

16. Baima Bollone, P.L., et al., "Ricerca degli antigeni M, N ed S nelle trace di sangue sulla Sindone", *Sindon* 34: 9-13 (1985).

17. Blumenfeld, O., "Non-human orthologs of the MNS blood group system", http://www.ncbi.nlm.nih.gov/projects/gv/mhc/xslcgi.cgi?cmd=bgmut/orthologs_mns (2011).

18. Rearden, A. et al., "Glycophorin B and glycophorin E genes arose from the glycophorin A ancestral Gene via two duplications during primate evolution", *J. Biol. Chem.* 268: 2260-2267 (1993).

19. Blumenfeld et al., *Molecular Biology and Evolution of Blood Group and MHC Antigens in Primates*, Springer-Verlag, Berlin, Germany (1997).

20. Pennisi, E., "And the gene number is…?" Science 288: 1146-1147 (2000).

21. Savatier, P. et al., "Nucleotide sequence of the Beta-globin genes in gorilla and macaque: The origin of nucleotide polymorphisms in human", M. Mol. Evol. 24: 309-318 (1987).

22. Barberis, B., "Perspectives for the future study of the Shroud", http://www.shroud.com/pdfs/barberisv.pdf, (2012).

23. Kearse, K.P., et al. "TCR alpha-CD3 delta epsilon association is the initial step in alpha beta dimer formation in murine T cells and is limiting in immature CD4+ CD8+ thymocytes", *Immunity* 2: 391-399 (1995).

24. Van Leeuwen, J.E.M., and K.P. Kearse, "Deglucosylation of N-linked glycans is an important step in the dissociation of calreticulin-class I-TAP complexes", *Proc Natl. Acad. Sci USA* 93: 13997-14001 (1996).

25. Osuchowski, M.F., et al., "Sequential ELISA to profile multiple cytokines from small volumes", *J. Immunol. Methods* 302: 172-181 (2005).

26. Osuchowski, M.F. and D.G. Remick, "The repetitive use of samples to measure multiple cytokines: the sequential ELISA", *Methods* 38: 304-311 (2006).

27. Independent Forensics, http://www.ifi-test.com/pdf/BloodValid.pdf (2010).

28. National Institutes of Health online bookshelf, http://www.ncbi.nlm.nih.gov/books/NBK2274/, (2005).

29. Marsh, S.G.E. et al., "Nomenclature for factors of the HLA system", *Tissue Antigens* 75:291-455 (2010).

30. National Institutes of Health online bookshelf, http://www.ncbi.nlm.nih.gov/books/NBK2270/, (2005).

31. Espinoza, E.O., et al., "Identification and quantitation of source from hemoglobin of blood and blood mixtures by high performance liquid chromatography", *J. Forensic Sciences* 41: 804-811 (1996).

32. Kimura, A., et al., "ABO blood grouping of bloodstains by sandwich ELISA using monoclonal antibody specific for human red cell band 3", *Int. J. Leg. Med* 105: 209-212 (1993).

33. Yaffe, M.P. "The machinery of mitochondrial inheritance and behavior", *Science* 283: 1493-1497 (1999).