DNA on the Shroud of Turin:
Distinguishing endogenous versus exogenous DNA

Abstract

In the late 1990s it was reported that human DNA existed on the Shroud of Turin, and although in a generally degraded state, certain regions were sufficiently intact to clone and sequence three genes from bloodstained fibers: human betaglobin, amelogenin X and amelogenin Y. An unknown variable in such studies is the extent of contamination by exogenous DNA, transferred to the Shroud by persons or objects that have come in contact with cloth. Indeed, the abovementioned genes are not exclusive to blood cells, but are also found within other cell types, including skin cells. Here, a simple experimental approach is described for distinguishing endogenous versus exogenous DNA, which may help establish that DNA in the blood areas of the Shroud of Turin originated from white blood cells (lymphocytes) present on the cloth.

Human DNA on the Shroud

In the 1990s, Garza-Valdes reported in the book "The DNA of God" the cloning and sequencing of three human gene segments from blood remnants on the Shroud: the betaglobin gene (which encodes a portion of the hemoglobin protein), located on chromosome 11; and the amelogenin-X and amelogenin-Y genes, found on the X and Y sex chromosomes, respectively (1).

One issue that persists in DNA analysis of objects that have been handled by numerous individuals is contamination. Garza-Valdez stated in his book, "*obviously there was the possibility of contamination and possibility that blood from someone other than the crucified victim happened to fall on the part of the Shroud from which the sample was taken. But it is certainly more likely that the blood came from the Man on the Shroud, rather than a bystander, in view of the fact that the sample was taken from the back of the head, from the area where the crown of thorns would have damaged the head of the victim*" (1). Unlike hemoglobin protein, whose expression is primarily restricted to red blood cells, hemoglobin (betaglobin) DNA is contained within essentially all cell types throughout the body, including skin cells. Realistically, a person would not have to bleed on the Shroud to transfer betaglobin DNA, but merely to touch it. Or to touch an object, which then came in contact with the cloth, or with threads taken from it. Or perhaps even to touch an object, which then comes in contact with an object that contacts the sample.

The average human being sheds approximately 400,000 skin cells per day, a portion of which contains DNA that may be transferred by contact, referred to as touch DNA (2-4). The vast majority of cells in the outermost layer of skin have undergone a process known as keratinization, which includes hardening and loss of their nuclei. Small amounts of DNA fragments are believed to be

constantly sloughed off of the outermost skin layer, which may be transferred upon contact. Numerous variables may affect the extent of touch DNA transfer, including sweat, temperature, humidity, time of contact, type of material, etc. Contact does not always result in DNA transferal; how long touch DNA may survive is unclear and unique to each object (2-5). The extent of contamination of the Shroud by exogenous DNA is unknown, but given the communal nature of the cloth in both its past and even more recent history, it is reasonable to speculate that DNA from numerous individuals may be present on the Shroud. Prior to the advent of molecular biology and the development of DNA amplification techniques, meticulous handling of the Shroud (relative to good practice in preventing DNA contamination) was not a primary consideration (Figure 1). While it is certainly possible that the cloned segments originated

Figure 1. Examination of the Shroud of Turin. *Examination of the cloth in 1978 by the Shroud of Turin Research Project (STURP) team.*

from blood cells, DNA contamination is an unknown variable that may confound the interpretation of these results (and perhaps certain future DNA studies; a similar "hands on" approach was used in the cutting of the cloth for C-14 experiments in 1988 and restoration/mending efforts in 2002, as evidenced by various photographs documenting these events). A determined skeptic might argue that contaminating DNA is responsible, a charge that would be somewhat difficult to refute.

Blood cells (lymphocytes) and DNA rearrangement
  Three major blood cell types are present within the body: erythrocytes (red blood cells), white blood cells, and cellular fragments termed platelets. In the human, red blood cells lose their nucleus as they mature; only white blood cells contain DNA. Approximately 35% of white blood cells are lymphocytes, which function as the body's major defense against foreign organisms and disease, known as the immune system (6,7).

Two major classes of lymphocytes exist, B lymphocytes, or B cells, which synthesize antibodies (immunoglobulin), and T lymphocytes, or T cells, that utilize a membrane bound receptor, termed the T cell receptor, or TCR (7), (Figure 2). B cells may also synthesize a secreted form of antibody
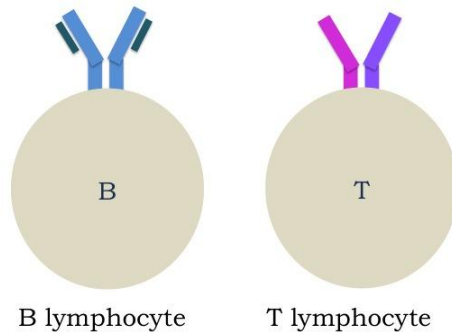

Figure 2. Antigen receptors expressed on B and T lymphocytes. *B cells express on their surfaces a membrane receptor consisting of two identical heavy chains (blue), joined with two identical light chains (green). The B cell receptor is termed antibody, or immunoglobulin. B cells may also synthesize a secreted form of the receptor (not shown). T cells express a membrane receptor consisting of a TCRα chain (pink) joined with a TCRβ chain (purple), termed the TCR. See text for details.*

(immunoglobulin) into the circulation. The TCR on T lymphocytes is exclusively a membrane bound surface receptor, composed of two different proteins, a TCRα chain joined with a TCRβ chain (Figure 2). Lymphocytes are unique in that unlike any other cell type in the body, they undergo somatic recombination during their maturation and development. More specifically, the genes encoding their surface receptors undergo rearrangement and splicing. DNA rearrangement is unique to lymphocytes and represents the molecular basis for the generation of the huge diversity of immune receptors that exist for virtually any antigen (foreign substance) that may enter the body (7). Although other cell types (e.g. skin cells) contain the genes for lymphocyte receptors, the DNA remains in the non-rearranged, or germline, configuration throughout the cell's lifetime.

In the formation of a functional antigen receptor in B cells, a so-called heavy chain gene and a light chain gene must undergo functional rearrangement and splicing; heavy and light chain genes are located on separate chromosomes (see Table 1 below). A somewhat simplified version of this process is shown in Figure 3, using one of the light chain genes (Ig-k) as an example. (Figure 3). This particular light chain gene contains 40 variable (V) segments, 5 joining (J) segments, and a single constant (C) segment, which are separated by significant distances from each other on the chromosome. During lymphocyte development, gene segments are recombined, creating a much smaller form of the DNA;
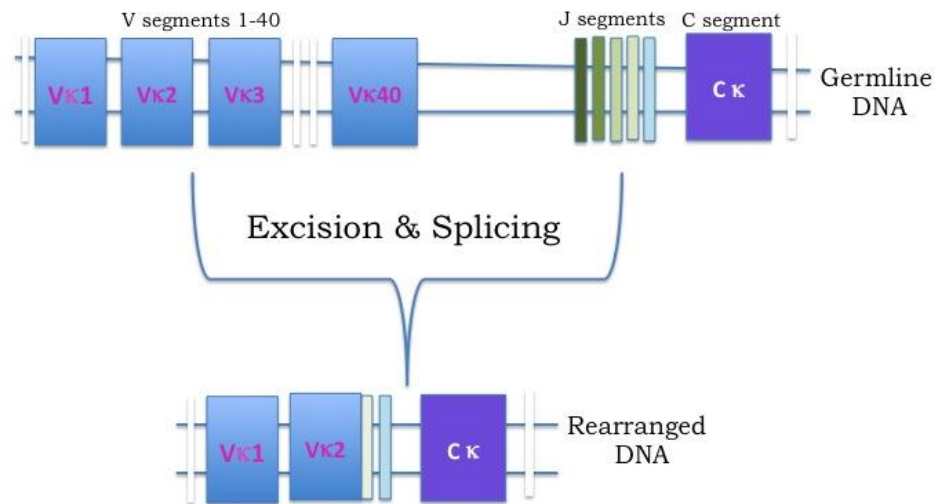
3

Figure 3. Recombination of the Immunoglobulin kappa light chain gene (Ig-k) as an example of DNA rearrangement in lymphocytes. In the formation of a functional receptor in B cells, DNA from three different types of gene segments, Variable (V), Joining (J), and Constant (C) are recombined and spliced together. Intervening sequences are excised and removed. See text for details.

intervening sequencing are excised and removed (7). The rearranged DNA will undergo further editing and processing into RNA, where only a single V, J, and C region will be joined together (not shown). Receptor diversity is achieved through the random combination of such gene segments at the DNA level. Similar gene rearrangement occurs for the Ig-heavy chain genes (in B cells) and the formation of the TCR in T lymphocytes (7). In non-lymphoid cells, such receptor genes would be present, but exist in the germline (unrearranged) configuration.

Analysis of Shroud DNA for lymphocyte gene rearrangement
The DNA on the Shroud is reported to be badly fragmented, although certain gene fragments were sufficiently intact for cloning and sequencing of betaglobin and the amelogenin genes (1). Because such studies were unable to distinguish DNA from endogenous (white blood cell) versus exogenous (skin cell) sources, it is important to consider an experimental approach that could differentiate between the two. Indeed, such studies might help establish that DNA from white blood cells (B and T lymphocytes) is present within the blood areas of the cloth.

The study and manipulation of DNA was greatly enhanced in the early 1980s by the development of a molecular biology technique known as the polymerase chain reaction, or PCR (8). The PCR method relies on repeated cycling of DNA

4

replication to exponentially amplify DNA, allowing even small gene fragments to be very rapidly and effectively analyzed (Figure 4). Indeed, PCR can be used to create approximately a billion DNA copies within a modest number of cycles, in just three hours time. Specific DNA regions are effectively targeted for study by using primers that correspond to the particular gene sequence(s) of interest (see Figure 5 below). Analysis of B cell and T cell gene rearrangement is a well-
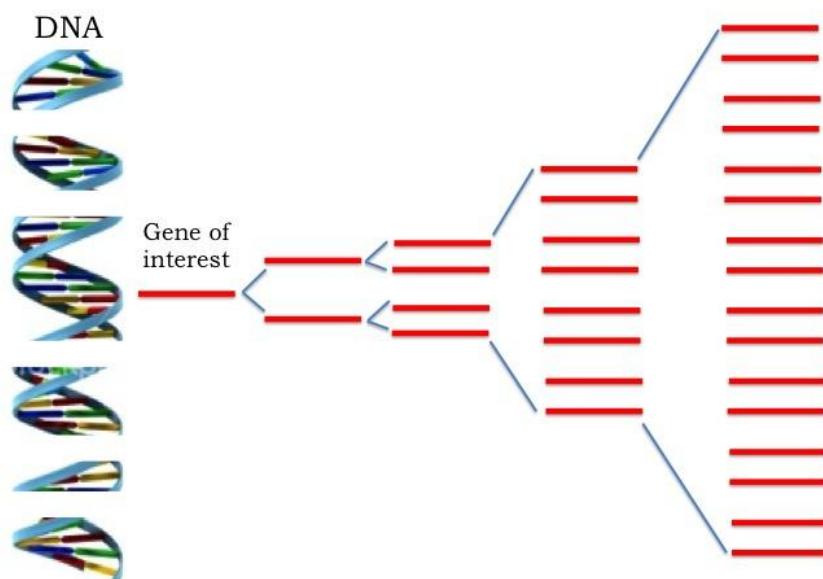

Figure 4. The Polymerase Chain Reaction Technique. *A specific gene of interest is copied and cyclically amplified, allowing very small amounts of DNA to be effectively analyzed in the laboratory. In 30 such cycles, over a billion copies of the gene are created. See text for details.*

established procedure in the diagnosis and evaluation of many lymphoproliferative disorders, including non-Hodgkin's lymphoma and T cell leukemias (9-12); because each lymphocyte contains a unique antigen receptor, PCR is useful in determining if a particular gene rearrangement is overrepresented in the general lymphocyte population, indicative of a lymphoma.

As shown in Table 1, multiple B cell and T cell receptor genes exist that could be suitable for DNA analysis (Table 1). Given the fact that many of these genes are present on different chromosomes might increase the chances for detection, if certain DNA regions are more fragmented than others. B cells are not normally found in the skin (13); small amounts of T cells exist within the epithelial layer, which may increase with inflammation and certain pathological

Table 1. B lymphocyte and T lymphocyte Receptor Genes

Gene	Chromosome	B cells	Other cells
Ig-H	14	Rearranged	Germline
Ig-κ	2	Rearranged	Germline
Ig-λ	22	Rearranged	Germline

Gene	Chromosome	T cells	Other cells
TCRα	14	Rearranged	Germline
TCRβ	7	Rearranged	Germline

Table 1. B lymphocyte and T lymphocyte receptor genes. *The chromosomal location of immunoglobulin heavy chain (Ig-H), immunoglobulin light chains (Ig-κ, Ig-λ), and T cell receptor (TCRα, TCRβ) genes are indicated. DNA rearrangement is specifically restricted to B and T lymphocytes; in all other cell types, the genes remain in the germline (unrearranged) configuration. See text for details.*

skin conditions (14-16). DNA Analysis of bloodstains should yield a profile indicative of a polyclonal population of B cells, e.g. a bell-shaped curve (Gaussian distribution) that reflects the heterogeneous nature of receptor rearrangement (9-11), (Figure 5). Contaminating DNA from skin cells (touch DNA), should show no product (Figure 5); in an unrearranged configuration, the gene segments remain large distances apart from each other on the chromosome, making primer extension unachievable. Lymphocytes are a relatively small proportion of white blood cells, with most (50-70%) being neutrophils, which do not undergo gene rearrangement (7). Importantly, because the assay is designed to detect the presence of rearrangement, the signal is expected to be above any background noise that may exist. Control (non-Shroud) bloodstains run in parallel would provide comparison profiles. For the Shroud, samples taken from several sites would yield the most definitive
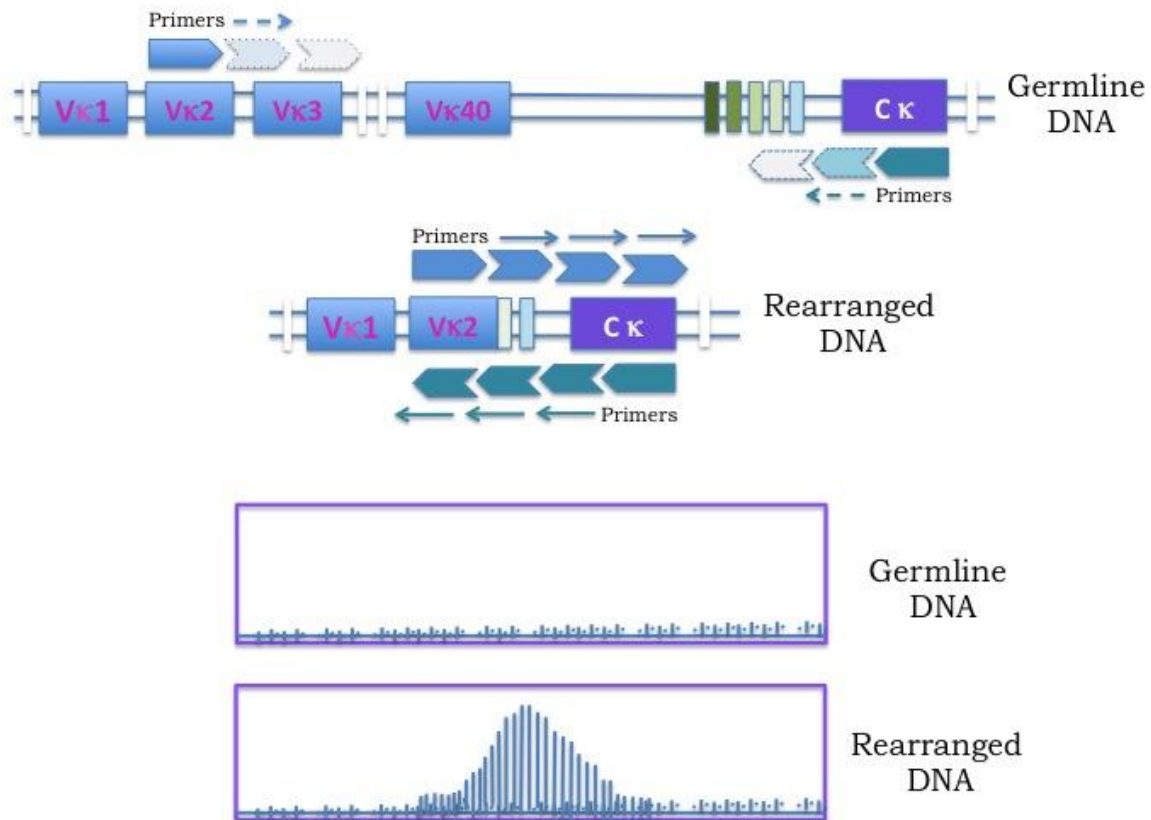
Figure 5. PCR analysis of lymphocyte receptor DNA (Ig-κ in this example) in germline and rearranged configurations. *A series of primers (master mix) is used to initiate the copying of specific DNA sequences, which are then replicated and cyclically amplified as previously shown in Figure 4. Unlike rearranged DNA, which is effectively copied and replicated, germline (unrearranged) DNA is not, as the genes are separated by large distances on the chromosome. The predicted outcome for germline and rearranged DNA is shown in the bottom panels. Germline DNA results in no specific product, showing only background fluorescence. A bell-shaped curve is observed in the amplification of rearranged DNA because the cell population is polyclonal, comprised of many (different) B cells, each of which utilizes a particular combination of genes in the creation of their receptor. See text for details.*

conclusion. Finally, it should be noted that the opportunity might exist for analysis of more than one type of receptor gene (see Table 1), depending on the condition of the DNA relevant to those gene segments.

Acknowledgements

References

1. Garza-Valdes, L., *The DNA of God?* Doubleday, New York, USA (1999).

2. Wickenheiser, R.A., "Trace DNA: A review, Discussion of Theory, and Application of the Transfer of Trace Quantities of DNA Through Skin Contact", *J. Forensic Sci.* 47: 442-450 (2002).

3. Van Oorscot, R.A.H. and Jones, M.K., "DNA fingerprints from fingerprints", *Nature* 387: 767 (1997).

4. Ryan, S.R., "Touch DNA. What is it? Where is it? How much can be found? And, how can it impact my case: A Question and Answer guide to all things Touch DNA",
http://www.ryanforensicdna.com/yahoo_site_admin/assets/docs/Touch_DNA_article.59101908.pdf, (2012).

5. Daly, D.J., et al., "The transfer of touch DNA from hands to glass, fabric, and wood", *Forensic Sci. Int. Genet.* 6: 41-46 (2012).

6. Flynn, J.C. *Essentials of Immunohematology*, W.B. Saunders, Philadelphia, PA (1998).

7. Janeway, C., et al. *Immunobiology*, Garland Science Publishing, New York, NY (2005).

8. Mullis, K.B., *The Polymerase Chain Reaction*, Birkhauser, Boston, MA (1994).

9. Williams, M.E., et al., "Immunoglobulin and T cell receptor gene rearrangements in human lymphoma and leukemia", *Blood* 69: 79-86 (1987).

10. Morales, A.V., et al., "Evaluation of B-cell clonality using the BIOMED-2 PCR method effectively distinguishes cutaneous B-cell lymphoma from benign lymphoid infiltrates", *Am J. Dermatopathol.* 30: 425-430 (2008).

11. Liu, H., et al., "A practical strategy for the routine use of BIOMED-2 PCR assays for detection of B- and T-cell clonality in diagnostic hematopathology", *Br. J. Haematol.* 138: 31-43 (2007).

12. Theriault, C., et al., "PCR analysis of Immunoglobulin Heavy Chain (IgH) and TCR-γ chain gene rearrangements in the diagnosis of lymphoproliferative disorders: Results of a study of 525 cases", *Mod. Pathol.* 13: 1269-1279 (2000).

13. Bos, J.D., et al., "The skin immune system (SIS): distribution and immunophenotype of lymphocyte subpopulations in normal human skin: *J. Invest. Dermatol.* 88: 569-73 (1987).

14. Clark, R.A., et al., "The vast majority of CLA+ T cells are resident in normal skin", *J. Immunol.* 176: 4431-4439 (2006).

15. Morris, A.M., et al., "T lymphocytes bearing the gamma-delta T-cell receptor: a study in normal human skin and pathological skin conditions", *Br. J. Dermatol.* 127: 458-62 (1992).

16. Bos, J.D., and Kapsenberg, M.L., "The skin immune system: progress in cutaneous biology", *Immunology Today* 14: 75-78 (1993).