

Discussion of a Recent Paper on Sporadic Inclusion Body Myositis:

Greenberg SA, Pinkus JL, Amato AA, Kristensen T, Dorfman DM Association of inclusion body myositis with T cell large granular lymphocytic leukaemia. *Brain*. 2016 May;139(Pt 5):1348-60. <http://brain.oxfordjournals.org/content/139/5/1348>

By Alexandra M. Levitt

The findings in a recent paper (1, also see 2) support the idea that the sporadic Inclusion Body Myositis (sIBM) is an autoimmune disease—an idea that has been in doubt because some drugs that suppress the immune system (and are used to treat other autoimmune conditions) have been ineffective in sIBM patients. Although sIBM is characterized by muscle inflammation associated with T cells, the presence of T cells in muscle fibers could be a response to whatever is causing the muscle weakness, rather than the original cause of the disease.

This paper suggests that the T cell invasion is, after all, the cause of sIBM—a conclusion that opens up new lines of research that could lead to new forms of treatment. The results also suggest possible new directions for sIBM diagnosis that involve blood tests rather than muscle biopsies.

The impetus for the study was the finding that two relatively rare diseases often occur together: sIBM and T cell large granular lymphocytic (T-LGL) leukemia:

- sIBM is a muscle disorder that involves the clonal expansion of cytotoxic (CD8+) T cells, possibly in response to a virus, although no specific viral infection has been identified. These T cells apparently recognize (and bind to) an unknown antigen or antigens on muscle fibers, causing the body's immune system to attack and damage its own tissues.¹
- T-LGL leukemia is a blood disorder that also involves the clonal expansion of cytotoxic (CD8+) T cells. The name “large granular lymphocytic leukemia” reflects the visual characteristics of the T cells, which are large in size and contain cytotoxic granules (i.e., substances that destroy cells). As they circulate in the blood, the large granular lymphocytes (LGLs) can damage blood cells and affect the spleen, the liver, and other parts of the body, becoming a systemic disease

Working on the hypothesis that the co-occurrence of two diseases involving clonal expansion of cytotoxic (CD8+) T cells is unlikely to be a coincidence, the authors looked for evidence of T-LGL leukemia in sIBM patients. They found that some 58% of sIBM patients meet the clinical criteria for T-LGL leukemia. Specifically, they found that:

- sIBM is frequently associated with expanded clonal populations of LGLs in the blood (not only in muscle). The absolute number of LGLs in the blood of some sIBM patients is as high as in T-LGL leukemia patients, and the abundance of muscle-invading LGLs correlates with the abundance of LGLs in the blood.
- There is a correlation between more aggressive sIBM disease and the presence of expanded clonal populations of LGLs in the blood.

¹ The clonal expansions of cytotoxic (CD8+) T cells that occur in sIBM and T cell LGL leukemia might be due to chronic antigen stimulation caused by persistent or chronic viral infection (3, 4). Another possibility is that once a virus has triggered a clonal expansion of T cells the virus is eliminated and the autoimmune response continues on its own (3).

- T cells from T-LGL leukemia patients are known to have a high rate of gene rearrangements in the non-specific parts of the T cell receptor gene (i.e., the parts that are not involved in protein binding to a specific antigen). This was also found to be true for T cells from sIBM patients.
- Known “biomarkers” of T-LGL leukemia are also found in the blood of sIBM patients. These include elevated levels of lymphocytes and decreased ratios of CD4 to CD8 T cells. These biomarkers might be useful in diagnosing cases of sIBM.

In discussing these results, the authors suggest that “in many patients with sIBM, the autoimmune process has evolved into a neoplastic-like process.” In other words, sIBM might initially develop as an autoimmune expansion of T cells (perhaps stimulated by a viral infection) that mistakenly recognize a “self-antigen” on muscle cells as a foreign, “non-self” antigen. In some patients, if this situation persists, the aberrant T cells may undergo further genetic changes that lead to the development of T-LGL leukemia. These changes would favor continued expansion and survival of LGLs, by allowing them to bypass the cellular controls that normally regulate their reproduction and lifecycle.

To follow up this idea, scientists could characterize T-LGL cells from sIBM patients in depth, to figure out how these cells differ from normally regulated T cells and why they are resistant to immunosuppressive drugs. These cells could be investigated at the molecular level by sequencing their genomes to look for mutations that affect regulatory pathways and by comparing patterns of gene transcription and protein translation. (The authors looked for mutations in a particular regulatory gene called STAT-3 but did not find changes in STAT-3 genes in T cells from sIBM patients.)

This research is encouraging, because it suggests a way to figure out what is going on and where to look for answers that could spur the development of new treatments for sIBM. One approach might be to identify the muscle antigen to which the T cells bind in sIBM patients (the antigen “flag”) and develop a monoclonal antibody treatment that prevents the T cells from binding. If successful, such a treatment might keep the illness from progressing and might also allow the muscles to recover.

Another approach is to identify new treatments for T-cell LGL leukemia that might also work for sIBM, assuming that both diseases are in fact caused by clonal expansions of CD8+ cells. One possibility is a monoclonal antibody treatment called alemtuzumab (5, 6) that binds to CD52, a protein present on the surface of mature lymphocytes (including LGLs), leading to the destruction of those cells. This treatment shows some effectiveness against a range of leukemias and has been tested as an sIBM treatment in an open-label trial. Although the results of that trial were reported as promising—suggesting that antibody infusions might slow disease progression for up to 6 months and improve the strength of some patients (7)—additional analysis is needed to validate the magnitude and statistical significance of the study’s outcome measures and to assess possible confounding factors and sources of assessment bias (8). Another issue concerns the potential side effects of alemtuzumab, which destroys many different types of immune system cells. In view of this issue, research is underway to develop and test a less-toxic monoclonal antibody treatment for sIBM that kills only a selected subpopulation of T cells (Dr. Steven A. Greenberg, personal communication).

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1. Greenberg, S. A., Pinkus, J. L., Amato, A. A., Kristensen, T., & Dorfman, D. M. (2016). Association of inclusion body myositis with T cell large granular lymphocytic leukaemia. *Brain*, 139, 1348–1360. <http://doi.org/10.1093/brain/aww024>
2. Hohlfeld, R., & Schulze-Koops, H. (2016). Cytotoxic T cells go awry in inclusion body myositis. *Brain*, 139, 1312–1314. <http://doi.org/10.1093/brain/aww053>

3. Dalakas, M. C., & Schmidt, J. (2016). Viruses in IBM: Hit-and-run, hide and persist, or irrelevant? *Neurology*, 86(3), 204–5. <http://doi.org/10.1212/WNL.0000000000002295>
4. Steinway SN, LeBlanc F, Loughran TP Jr. The pathogenesis and treatment of large granular lymphocyte leukemia. *Blood Rev.* 2014 May;28(3):87-94. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4155502/>
5. Osuji N, Del Giudice I, Matutes E, Morilla A, Owusu-Ankomah K, Morilla R, Dunlop A, Catovsky D. CD52 expression in T-cell large granular lymphocyte leukemia--implications for treatment with alemtuzumab. *Leuk Lymphoma.* 2005 May;46(5):723-7. <http://www.ncbi.nlm.nih.gov/pubmed/16019510>
6. Mohan SR, Clemente MJ, Afable M, Cazzolli HN, Bejanyan N, Wlodarski MW, Lichtin AE, Maciejewski JP. Therapeutic implications of variable expression of CD52 on clonal cytotoxic T cells in CD8+ large granular lymphocyte leukemia. *Haematologica.* 2009 Oct;94(10):1407-14. <http://www.ncbi.nlm.nih.gov/pubmed/19794084>
7. Dalakas MC, Rakocevic G, Schmidt J, Salajegheh M, McElroy B, Harris-Love MO, Shrader JA, Levy EW, Dambrosia J, Kampen RL, Bruno DA, Kirk AD. Effect of Alemtuzumab (CAMPATH 1-H) in patients with inclusion-body myositis. *Brain.* 2009 Jun;132(Pt 6):1536-44. <http://brain.oxfordjournals.org/content/132/6/1536.long>
8. Greenberg SA. Comment on alemtuzumab and inclusion body myositis. *Brain.* 2010 May;133(Pt 5):e135; author reply e136. <http://brain.oxfordjournals.org/content/133/5/e135.long>

A one paragraph overview of sIBM.

By Bill Tillier

In sIBM an immune system reaction occurs, presumably triggered by antigen flags that appear on the surface of the muscle cells. What might cause the production of these antigen flags is unknown, possibly an undetected virus. The muscle cell becomes surrounded by **cytotoxic (CD8+) T cells** (CTLs) that attack and invade the muscle cell, killing it. These **cytotoxic (CD8+) T cells** (CTL) expand clonally, meaning that hundreds of millions of these cells flood the muscles and blood. This expansion continues over long periods of time, suggesting two possibilities: either a persisting, undiscovered infection causes an ongoing immune response, or an unknown virus infection initially triggers the development of autoimmunity – an ongoing situation where the immune system mistakenly attacks normal muscle. If the antigen “flag” for sIBM can be discovered and characterized, it would likely lead to the development of new treatment strategies. If this “new” model is correct, the other abnormalities commonly seen in sIBM, such as protein degeneration, would be secondary to (and likely caused by) these immune system problems.

Priority questions.

By Bill Tillier

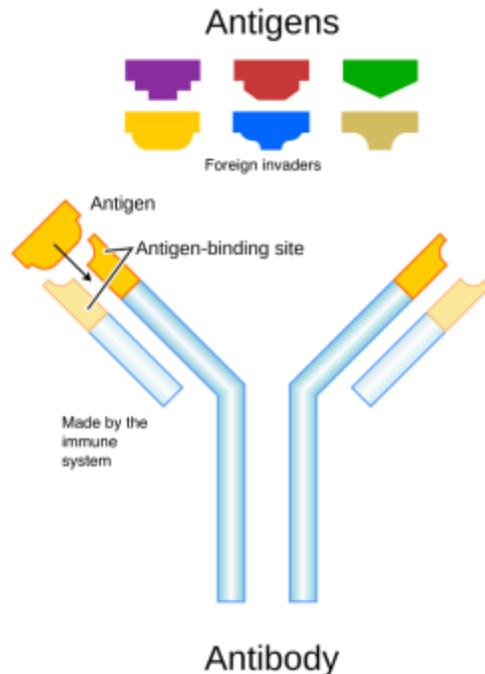
- ◆ What causes the clonal expansion of **cytotoxic (CD8+) T cells**?
- ◆ How can the clonal expansion of **cytotoxic (CD8+) T cells** be stopped?

- ◆ Is there a persistent and ongoing viral infection that causes the muscle cell to display antigens, continually triggering destruction by the immune system (Dalakas and Schmidt, 2016)?
- ◆ If an autoimmune response has been generated, what triggered it? A virus that “hit and ran” (Dalakas and Schmidt, 2016)?
- ◆ Can the antigen “flag” presumably displayed by the muscle cells be discovered and characterized?
- ◆ Once the antigen “flag” is understood, can monoclonal antibodies be generated to block it? Would this strategy help the muscles to recover?

A brief glossary of terms and concepts.

By Bill Tillier

- ◆ sIBM: sporadic inclusion body myositis
- ◆ Self: referring to chemicals and structures normally belonging to the self – these are normal structures.
- ◆ Non-self: referring to chemicals and structures that should not be present, for example, viral or bacterial infections of the body.
- ◆ Cytotoxic: Anything that kills a cell.
- ◆ Non-necrotic: healthy
- ◆ Leukocyte: White blood cells, including lymphocytes, granulocytes, monocytes, and macrophages. A leukemia can involve any of these cell types.
- ◆ Immunity: Protection against disease created by the specialized cells and parts of the immune system.
- ◆ Autoimmune: Disease caused by antibodies or lymphocytes mistakenly produced against “self” (substances that are naturally part the body).
- ◆ Antigen: Any substance that causes an immune system to produce antibodies against it. Common foreign invaders are bacteria, viruses, fungi and parasites. Infected cells display antigens (“flags”) on their surface reflecting the specific chemical makeup and shape that characterizes the infection.
- ◆ Antibodies: When an antigen flag is detected, the immune system creates custom antibodies – uniquely structured and shaped proteins that correspond to the structure and shape of the antigen. This way, like a lock and key, an antibody will lock into the specific antigen involved.



◆ T lymphocytes (T cells): These special cells are designed to kill infected cells within the body – they are cytotoxic – they kill the cells they target. They are a type of white blood cell (Leukocytes) and are extremely important in the immune system.

◆ T cell receptor (TCR): The T cell receptor or TCR is a clump of proteins found on the surface of T lymphocytes (T cells) that recognize antigen flags. This recognition causes the activation of the T cell that subsequently attaches to, and kills, the recognized cell.

◆ Antigen-specific T cell receptor (TCR): In this case, the receptor is looking for a very specific flag and only this specific flag will be recognized.

◆ Cytotoxic (CD8+) T cells: (also called CTL cells): A T cell with a CD8 receptor (a bit of chemical on the cell's surface) that recognizes antigens (usually associated with viruses) on the surface of an infected cell — the cytotoxic (CD8+) T cell binds onto the infected cell and kills it.

The growth or division of cytotoxic (CD8+) T cells normally is temporary, occurring in response to a viral antigen on the surface of an infected cell. After the infection has been cleared up, the growth of the T cells normally stops.

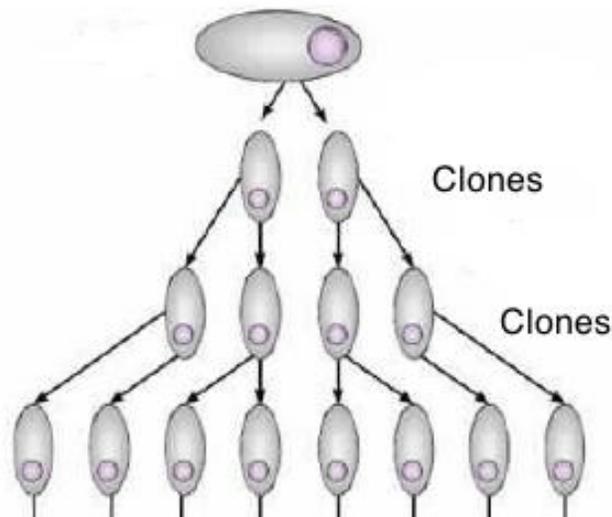
◆ Clonally expanded: Clonal expansion is characterized by the production of millions of daughter cells all arising originally from one single cell. Each new daughter cell is genetically identical to the first cell.

In a clonal expansion of lymphocytes, all progeny will lock onto the same antigen.

In leukemias, T cells or other white blood cells continue to divide and grow after the original immune threat is gone. This apparently occurs because these cells escape the normal mechanisms designed to shut down division and growth once an infection has been dealt with.

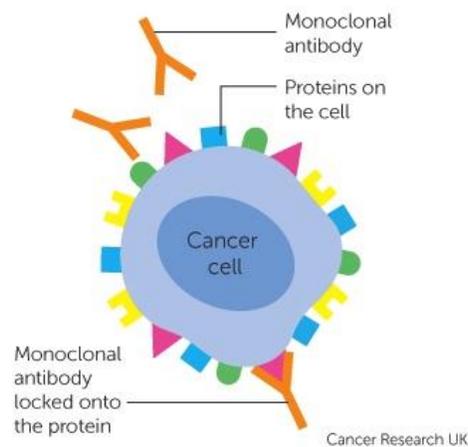
In sIBM cytotoxic (CD8+) T cells expand clonally.

Clonal expansion is a key characteristic of cancer and one of the major obstacles faced in treating cancer.



◆ Monoclonal: A clone from a single individual or cell. A popular term for the type of clonal expansion described above.

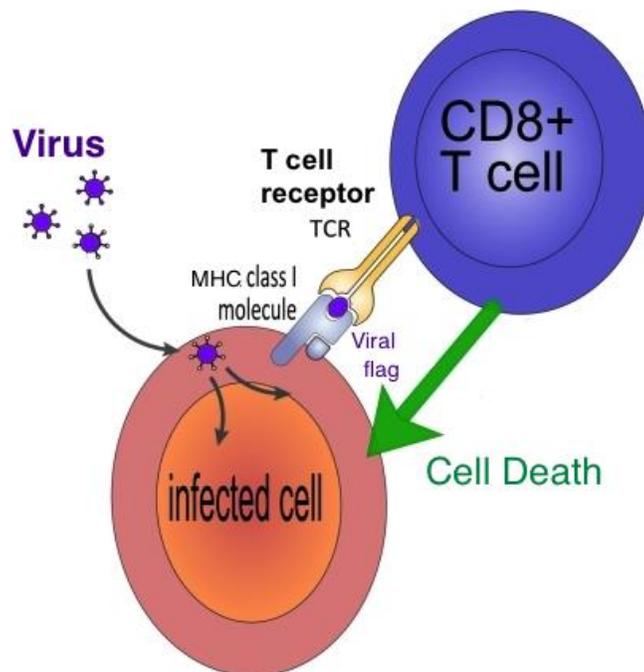
◆ Monoclonal antibodies: A new treatment strategy currently in use for cancer. For this treatment, the antigen flag must be known – described in terms of its chemical structure and shape. In this way, a monoclonal antibody can be manufactured in a research laboratory specifically to fit into the antigen flag. There are two main ways this can help; locking onto the antigen flag can help attract the immune system to react and kill the cancer cell. Alternately, once the treatment antibody has attached to the antigen, it will block it, preventing cytotoxic (CD8+) T cells from latching on.



This blocking strategy could be a potential treatment for sIBM. If the sIBM antigen flag can be discovered and understood, a monoclonal antibody could be manufactured in the laboratory to lock into it.

Once locked into, this antigen flag is blocked and can no longer be used by the cytotoxic (CD8+) T cells to target the muscle cell for destruction. Flooding muscle with monoclonal antibodies specifically designed to block the antigen might reduce the effectiveness of the cytotoxic (CD8+) T cells' attack and slow the destruction of muscle cells.

◆ Major histocompatibility class I (MHC I +): MHC I molecules are found on the surfaces of all cells and work to display bits of the different proteins being made within a cell. A normal cell will display flags showing pieces of the normal proteins contained within it ("self") and the immune system will recognize "self" and not respond. When a cell contains foreign ("non-self") proteins, usually caused by viral infection, some of the MHC I molecules will display these "non-self" flags. These "non-self" flags attract the attention of the immune system. The immune system then brings in cells (cytotoxic (CD8+) T cells and other cells) to kill the infected cell.



◆ Immune system surveillance: The immune system is constantly watching the surfaces of cells for both self-flags, that it normally ignores, and foreign flags indicating infected cells that the immune system targets for destruction.

A very brief and simple overview of the immune system.

(based on <http://kidshealth.org/en/parents/immune.html>)

The immune system is made up of a network of cells, tissues, and organs that work together to protect the body. One important type of cells involved are **white blood cells**, also called **leukocytes**, which come in two basic types — **phagocytes**: cells that destroy invading organisms, and **lymphocytes**: cells that allow the body to remember and recognize previous invaders and help the body destroy them. **Phagocytes** and **lymphocytes** work together to seek out and destroy disease-causing organisms or substances.

Leukocytes are produced or stored in many locations in the body, including the thymus, spleen, and bone marrow. For this reason, they're called the lymphoid organs. There are also clumps of lymphoid tissue throughout the body, primarily as lymph nodes, that house the **leukocytes**.

Leukocytes circulate throughout the body between the organs and nodes via lymphatic vessels and blood vessels. In this way, the immune system works in a coordinated manner to monitor the body for germs or substances that might cause problems.

A number of different cells are considered **phagocytes**. The most common type is the neutrophil, which primarily fights bacteria. Other types of **phagocytes** have their own jobs to make sure that the body responds appropriately to a specific type of invader.

The two kinds of **lymphocytes** are **B lymphocytes (B-cells)** and **T lymphocytes (T-cells)**. **Lymphocytes** start out in the bone marrow and either stay there and mature into **B cells**, or they leave for the **thymus gland**, where they mature into **T cells**. **B-cells** and **T-cells** have separate functions: **B-cells** are like the body's military intelligence system, seeking out their targets and sending defenses to lock onto them. **T cells** are like the soldiers, destroying the invaders that the intelligence system has identified.

When **antigens** (foreign substances that invade the body) are detected, usually on a cell's surface, several types of cells work together to respond. **B-cells** are triggered to create customized **antibodies** that are uniquely structured and shaped proteins that correspond to the structure and shape of specific antigens. This way, like a lock and key, the antibodies will fit into the specific antigen involved.

Once produced, these antibodies stay in a person's body. If his or her immune system encounters that same antigen again, the appropriate antibodies are already there and can be quickly reactivated to do their job a second time. So, if someone gets sick with a certain disease, like chickenpox, that person usually won't get sick from it again.

This is also how **immunizations** prevent certain diseases. An immunization introduces the body to a small amount of antigen that is usually either very weak or even dead — this will not make someone sick, but is enough to allow the body to produce antibodies against that antigen. In this way, if the person is then exposed to this disease or antigen in the future, their immune system will be able to quickly react to combat or even prevent the illness.

Although antibodies can recognize an antigen and lock onto it, they are not capable of destroying it on their own. That's the job of the **T cells**, a part of the immune system designed to destroy cells showing antigens. **T cells** also are involved in helping signal other cells (like phagocytes) to do their jobs.

Immune system

