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Mass of Saint Gregory: Lymphocyte Transformation Tests as a Diagnostic Tool in Clinical Immunology

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Introduction

The painting The Mass of Saint Gregory, by Jheronimus Bosch (Figure 1) is based on a legend from the late middle Ages [1]. This legend deals with the theological question whether during consecration, bread and wine really are transformed into the body and blood, respectively, of Jesus Christ. Pope Gregory the Great, when celebrating mass, prayed to God for assistance because he wanted to convince two pagans attending his mass. The pagans were in doubt about the events which would take place during consecration. According to the legend, God permitted Saint Gregory a view of the resurrected Christ with his wounds. Christ's blood poured into the chalice and the host (the bread) was infused with the body of Christ. The two onlooking pagans were converted on the spot. There is however another version of the legend in which it is Saint Gregory himself who has doubts on the transformation. In still another version, a woman who baked the bread used as host couldn't believe that it could be transformed into the body of Christ. Neither the transformation of wine into blood, nor that of bread into human flesh, can readily be explained in strict biological terms, but for a religious belief that also isn't needed. In biology however, a different type of transformation, involving cells, in particular lymphocytes, is being used in research and for in vitro diagnostic procedures. The medical and biological aspects of that type of transformation, including the history as well as current and future applications, will be discussed in biological terms in this paper.

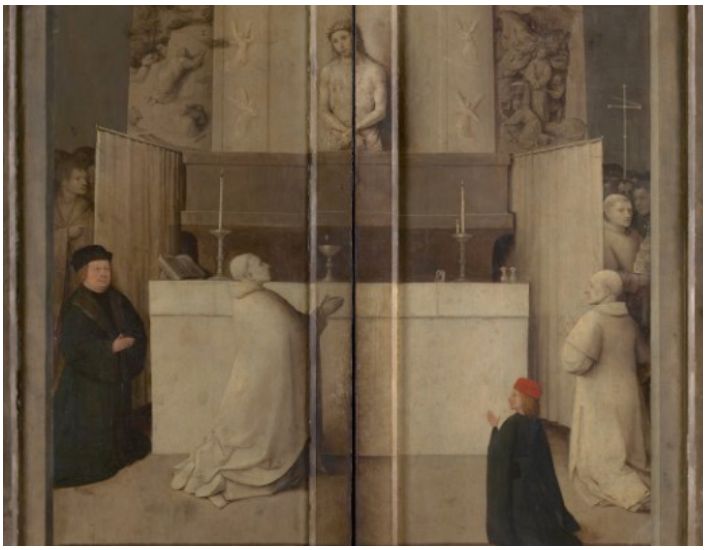

Figure 1: Saint Gregory's Mass (Triptych of the Adoration of the Magi, closed), Jheronimus Bosch, approximately 1510. Https://commons.wikimedia.org/wiki/File:Hieronymus_Bosch_062.jpg (assessed October 9, 2020).

Lymphocyte transformation by lectins

The first, and for a long time the most common compound used for in vitro lymphocyte transformation is Phytohemagglutinin (PHA). High concentrations of PHA are found in beans (especially

Phaseolis vulgaris, red kidney beans; see Figure 2), which, when eaten raw, can be toxic. PHA is composed of 2 subunits, E and L, of which PHA-E can agglutinate erythrocytes (E). PHA-E was used first in medicine for agglutination of red blood cells [2] and this is why it is called a haemagglutinin. The PHA-L subunit binds to the cell membrane of lymphocytes (see below). An alternative name for (plant) hemagglutinins is lectin, derived from the Latin word legere, meaning to select [3]. The reason for using the term for select is that the capacity of certain plant agglutinins to bind to erythrocytes was dependent on the blood group. Later on the term lectin was used in general, irrespective of blood group specificity.

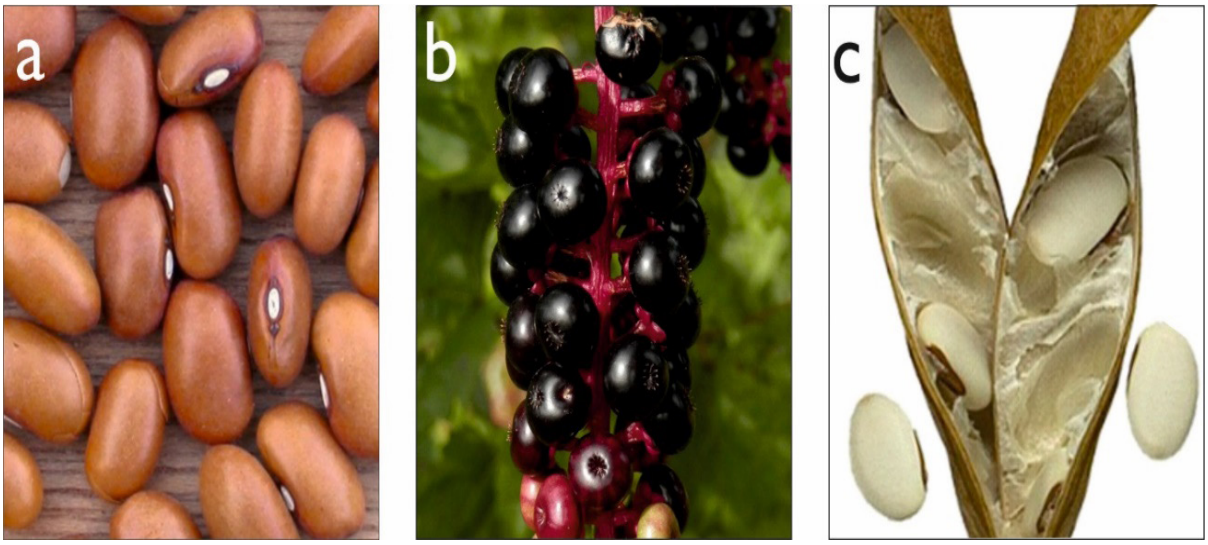

Figure 2: Botanical origin of common lectins used for in vitro lymphocyte transformation.

Phytohemagglutin from the beans of *Phaesolus vulgaris* (panel a), pokeweed mitogen from the berries of *Phytolacca americana* (panel b) and Concanavalin A from the beans of *Canavalia ensiformis* (panel c). Sources and credits Mr. Rasbak, https://commons.wikimedia.org/w/index.php?curid=1677401 (panel a), Ryan Hodnett, https://commons.wikimedia.org/wiki/File:American_Pokeweed_(Phytolacca_americana)_-_Kitchener,_Ontario_04.jpg (panel b), and Forest and Kim Starr, https://commons.wikimedia.org/wiki/File:Canavalia_ensiformis_MHNT.BOT.2013.22.55.jpg (panel c). All sources assessed on June 6, 2020.

Dr. Peter Nowell was the first one to demonstrate that PHA was able to induce proliferation of human lymphocytes [4]. Two versions of this discovery exist. The first one is that Nowell wanted to investigate cell proliferation in leukemia. When he had planned to take blood from a leukemia patient he found out (to his disappointment) that on that particular day the patient had gone into remission. He took the blood anyway, purified the lymphocytes by removal of the erythrocytes with PHA and to his surprise the lymphocytes started to grow and divide in vitro. When he repeated the experiment with his own blood, and that of his technician, he found that also normal lymphocytes could be stimulated to proliferate by PHA. The other version of the accidental discovery is that on a day in the lab he had started to purify lymphocytes from blood by adding PHA, but because he had to rush home for a family event, and because of that had forgotten to centrifuge the blood afterwards. The next morning he saw that the lymphocytes had undergone blastogenesis [5]. Up till the discovery of Nowell, the prevailing paradigm in immunology was that lymphocytes are cells at the end-stage of their lifespan, unable to proliferate or differentiate any further. His paper was eventually published by Cancer Research [4], but one of the reviewers thought the paper was "interesting . . . but of no conceivable significance to science" [6]. After a lag time of several years, the technique to induce in vitro lymphocyte proliferation by stimulation with PHA became a major laboratory tool in biomedical research and diagnostics (Figure 3). On December 31, 2020 PubMed lists 22,212 papers with the key word phytohemagglutinin [https://pubmed.ncbi.nlm.nih.gov/?term=phytohemagglutinin&sort=date ; see also Figure 1). Soon after the discovery that PHA was a powerful inducer of in vitro lymphocyte transformation, other plant lectins were discovered with properties, the most important being concanavalin A and pokeweed mitogen.

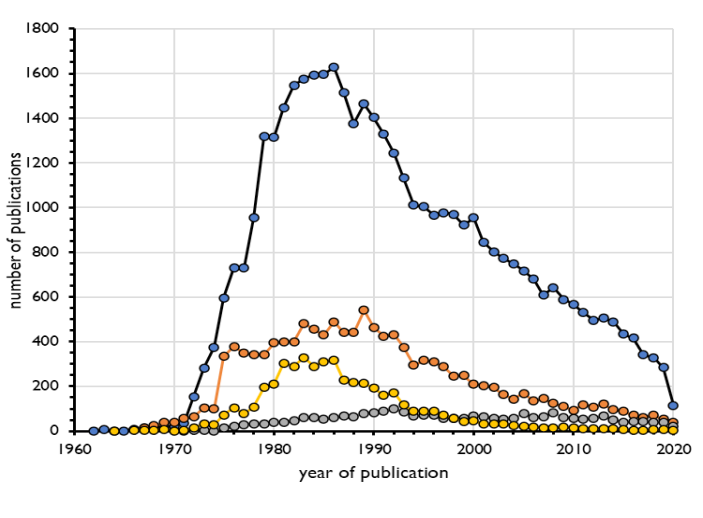

Figure 3: Overview of sixty years of biomedical research and publications on lymphocyte transformation with mitogens. Data were retrieved from PubMed (https://pubmed.ncbi.nlm.nih.gov/) using the search terms "lymphocyte" and "mitogen" (blue symbols), "PHA" (orange symbols), "ConA" (yellow symbols) or "pokeweed" (grey symbols). Data from 2020 span the period January-October.

Concanavalin A

A century ago, James Sumner isolated and crystalized a protein from jackbean, *Canavalis ensiformis* (Figure 2, panel c) which precipitated by half saturation with ammonium sulphate and was called concanavalin [7]. Upon dialysis, bisphenoid crystals precipitate first (Concanavalin A), precipitates in the form of needles last (Concanavalin B). Concanavalin A (ConA) binds to α-D-mannosyl and α-D-glucosyl sugar moieties. The biological activities of ConA are manifold [8,9]. In vivo, Con A has both proinflammatory as well as anti-inflammatory activity, depending on dosage and route of administration. Both in vitro and in vivo ConA has a vasorelaxation effect. For several tumor cell lines, ConA, because of its ability to induce apoptosis and autophagy, has antiproliferative effects. For selected species, ConA has antibacterial and antifungal activities. In the context of this manuscript, the most relevant biological activity of ConA is its mitogenic property for lymphocytes. This activity is directly related to the carbohydrate specificity of ConA, because the α-D-mannosyl and α-D-glucosyl moieties are (also) present on T cell receptor molecules [8,9]. Because ConA is a tetramer, the binding to T lymphocytes can cause extensive cross-linking of T cell receptors. Unlike other lectins, ConA binds with high affinity to cross-linked dextrans, such as Sephadex G-50. This allowed Mackler et al. to prepare "ConA-free" supernatants of lymphocytes cultured with ConA. These so-called ConA-supernatants contain lymphokines, which by themselves were mitogenic for autologous and allogeneic lymphocytes [10]. Later studies demonstrated that IL-2 was the major cytokine responsible for this activity. Cross-linking of T cell receptors by Con-A induces a rapid, transient increase in the cytoplasmic Ca^{2+} concentration, one of the first signs of cell activation [11,12] (Figure 4).

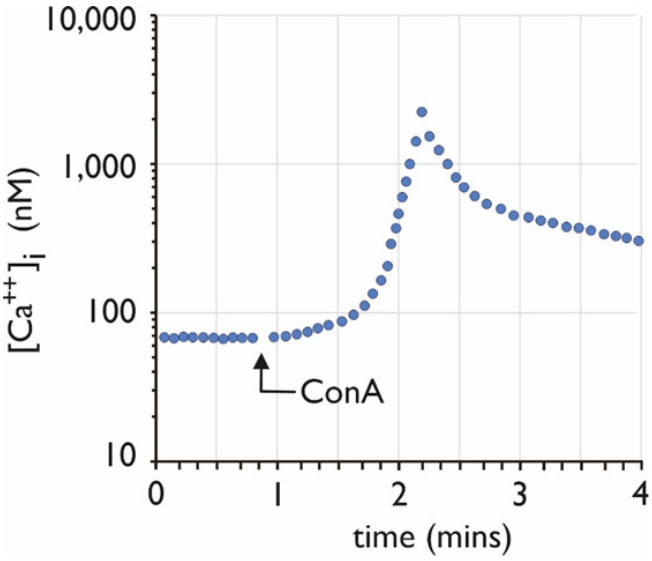

Figure 4: Kinetic analysis of changes in $[Ca^{++}]_I$ in human lymphocytes isolated from peripheral blood stimulated with ConA. Cells were kept at 37 ˚C during the experiment. The moment of addition of 10 µg/ml ConA is indicated with an arrow. Individual cells were measured continuously by flow cytometry; average values during a 6 seconds period are shown. Based on reference [12].

Pokeweed mitogen

The discovery of the biological properties of Pokeweed Mitogen (PWM), like that of PHA as described above, also has been accidental. Drs. Patricia Farnes and Barbara Barker were the pediatricians who discovered in 1961 that a fatal mysterious brain disease in a 3-year old girl was caused by ingestion of berries of pokeweed. When working in the laboratory to study and purify the active compound, now known as pokeweed mitogen, both doctors were exposed accidentally themselves leading to plasmacytosis in peripheral blood [13,14]. This led to the realization that PWM not only is a powerful inducer of T- and B-lymphocyte proliferation, but also of B-lymphocyte differentiation into plasma cells [15]. Pokeweed, *Phytolacca americana* (Figure 2, Panel b) in its raw form is highly poisonous. When properly cooked it can be eaten as a sallet [16]. Locally, the words sallet and salad are often used intermixed, a dangerous habit because when eaten raw, as when preparing a salad, pokeweed can be lethal. Tony Joe White wrote a song about a poor woman, Poke Salad Annie, who knew the recipe how to safely prepare a meal with pokeweed [17].

In vitro lymphocyte transformation, induced by either PHA, ConA or PWM, can be measured by the degree of proliferation, i.e. the net increase in number of cells as determined by counting the number of viable cells after a given culture period. The downside of such an approach is that during the culture period cells, especially the ones not taking part in the process, may die. Therefore, the net number of cells after culture actually can be lower than the starting number. For that reason most often ^{3}H thymidine incorporation is used as a proxy for cell proliferation [18]. The method is sensitive, can be semi-automated to a certain degree, but requires substantial laboratory infrastructure, including facilities to work with radioisotopes.

The complex biological nature of plant derived mitogens, and consequently batch to batch variations, have made most of these substances go out of fashion as of the 1990s (Figure 2). Moreover, the specific cellular ligands, being glycosylated surface proteins, have been difficult to characterize. For PHA they include the T cell receptor associated CD3 molecules as well as CD2 [19,20]. The decline of the use of plant lectins for in vitro lymphocyte proliferation was also caused by the availability of better standardized stimuli in the form of monoclonal antibodies directed against cell surface molecules on lymphocytes. Currently, polyclonal in vitro lymphocyte activation is being done with (mixtures of) monoclonal antibodies, e.g. the combination of anti-CD3 and anti-CD28 antibodies [21,22].

T lymphocyte activation by antigens and superantigens

When assessing the functional capacity of T lymphocytes in vitro, antigen-specific activation would be preferred over polyclonal activation with plant lectins or monoclonal antibodies against T cell receptors. However, in most cases the frequency of antigen-specific lymphocytes in unprimed individuals is too low to be measured in a quantitative manner. As an intermediate between polyclonal activation by mitogens and antigen specific activation, as described above, in vitro lymphocyte transformation can also be induced by so-called superantigens. Bacterial superantigens are produced by *Staphylococcus aureus* and *Streptococcus pyogenes* and are the causative agents of the toxic shock syndrome. The reason is that these superantigens can induce a massive cellular immune response and subsequent cytokine storm. Superantigens associate with HLA molecules and T cell receptors, outside of the peptide binding groove, and by crosslinking both molecules can cause activation of up to 20% of all T lymphocytes [23,24]. This effect is further enhanced by their binding to the co-stimulatory molecules CD28 and CD86 [25,26].

Specific antigens, such as peptides from Epstein Barr virus, cytomegalovirus [27], influenza virus [28] and yellow fever virus [29,30] can be successfully used for in vitro lymphocyte activation, provided the donors have been previously exposed to or infected by the respective viruses. Tetanus toxoid also is frequently used as an antigen-specific stimulus for lymphocytes from primed individuals [31].

In vitro lymphocyte transformation induced by either plant lectins, monoclonal antibodies, superantigens or specific antigens is an important component in the assessment of the functionality of the immune system. In vitro lymphocyte transformation tests are also being used for a number of other applications, as will be described below.

Lymphocyte transformation tests for assessment of hypersensitivity and for immunomodulatory medicines

The lymphocyte transformation test is useful in vitro tool for diagnosis of a number of hypersensitivity reactions against many different drugs, including antibiotics such as sulfadiazine, acenocoumarol, terbinafine, and beta-lactam antibiotics [32-34]. The LTT also is being used to detect nickel sensitization [35]. The outcome of the in vitro test cannot always be translated directly to the clinical situation, especially because a negative test doesn't rule out a drug hypersensitivity. Also used for screening or treatment of patients with metal sensitivity before temporomandibular joint replacement surgery [36].

The immunomodulatory activities of medicines such as cyclosporine, methotrexate, rapamycin, and dexamethasone can be studied using human in vitro lymphocyte activation assays. It has been demonstrated that these substances can cause dose-dependent inhibition of lymphocyte proliferation at concentrations within the therapeutic window [37]. In order to facilitate extrapolation of the obtained data to the clinical situation, the type of assays are performed with defined influenza peptides and lymphocytes from recently vaccinated donors. Using defined protocols, the IC_{50} of cyclosporine and mycophenolic acid as determined in five individual laboratories was found to be within an acceptable range [37].

Alternative techniques for assessment of lymphocyte activation

There are many alternatives to ^{3}H thymidine incorporation, including measurement of activation markers on cells and in vitro cytokine production, but few of them are equivalent of cell proliferation. Yet, the in vitro lymphocyte transformation tests, despite the use of standardized protocols, remains rather complicated and technically demanding [32]. A number of technical less demanding alternatives have been tested and developed which will be discussed below.

CFSE

Carboxyfluorescein Diacetate Succinimidyl Ester (CFSE) is a fluorescent dye, capable of covalently binding to intracellular molecules. Upon each cell division, the fluorescent molecules are

equally divided between each daughter cell, causing a reduction in fluorescence intensity per cell by approximately 50%. This reduction in fluorescence intensity can be measured by flow cytometry, and as a direct measure of the rate of cell division [38,39]. CFSE is especially effective in T lymphocyte activation studies because of its very low cell toxicity. The technique even can be used for antigen specific T-lymphocyte activation studies.

Interferon γ release assays

Another technique that is capable to measure antigen-specific lymphocyte activation in vitro is the so-called Interferon-Gamma Release Assay (IGRA) [40]. IGRA is a whole blood tests used to diagnose both latent as well as active Tuberculosis (TB) infections. White blood cells of TB infected patients release interferon γ (IFN-γ) upon in vitro (re-)activation with a mixture of specific antigens from *Mycobacterium tuberculosis* (ESAT-6, CFP-10, and TB7.7). The test includes a tube with PHA as a positive control and a negative control tube. Following incubation at 37 ˚C for 24 hrs, the concentration of IFN-γ in the culture supernatant is determined. The test has a high degree of sensitivity and specificity, but cannot distinguish between latent and active TB.

BrdU incorporation

5-bromo-2-deoxyuridine (BrdU) is a non-radioactive thymidine analog which can be used to detect in vitro T-lymphocyte proliferation. Furthermore, activated cells in S-phase of the cell cycle can be detected using flow cytometry with fluorescent labeled anti-BrdU antibodies, which can also be used as an alternative for measure of proliferation. This method has the added advantage that it can be combined with cell surface staining, allowing to distinguish between CD4 and CD8 T-lymphocyte activation [41].

Ki-67

Ki-67 is a protein that is widely used as a proliferation marker for human tumor cells. Ki-67 is expressed in both interphase and mitotic cells, but is upregulated dramatically during cell cycle progression [42]. It is equally sensitive as CSFE, or the CSFE-derivative Oregon Green, and more sensitive than BrdU in detection of in vitro tetanus toxoid specific T-lymphocyte activation. Using a combination of intracellular staining with fluorescent anti-Ki-67 and cell surface phenotyping, flow cytometric analysis can distinguish between CD8 and CD4 T-lymphocyte activation by PPD or methylisothiazolinone [43,44].

Cyclic voltammetry assay

Cyclic voltammetry is a technique that does not rely on the use of any labels for measuring in vitro lymphocyte transformation. Proliferating cells show specific voltametric behavior, which is the current passed through the solution in response to an applied potential. The short duration of 4 hours in combination with the ability to use fresh blood allows for an accurate indication of how cells would respond in their normal environment [45].

Conclusions and future perspectives

Assessment of the cellular and humoral immune status in health and disease requires a set of diagnostic techniques with high sensitivity and specificity. In vivo techniques (Latin for "within the living") such as a delayed type hypersensitivity reaction are only applicable for a limited number of indications (e.g. TB diagnostics). In this manuscript the in vitro techniques (Latin for "within the glass") for measuring lymphocyte transformation have been discussed. Especially in PHA stimulated lymphocytes, chromosomes are easily visible in metaphase, and therefore it also became the standard method in karyotyping for clinical genetic diagnostics [46]. For a (fortunately, limited) period PHA has been used in vivo as a form of treatment of aplastic anemia. The rationale was that "transformed" lymphocytes would thus acquire hematopoietic properties [47,48]. PHA also has been used for treatment of squamous cell carcinoma in a single study of 24 patients [49]. It soon became clear that the use of the lectins PHA, ConA, and PWM, because of their toxicity [13,14] should be restricted to in vitro applications. In vivo immunotherapy of malignant and other diseases requires better defined compounds, and monoclonal antibodies have been demonstrated to be highly effective in that respect.

In the future, techniques for immune status assessment could be developed, based on organoids of lymphoid organs, which would be closer to the in vivo situation. The term "ex vivo" has been proposed for such an approach [50,51].

Saint Gregory's Mass addresses the transformation which takes place during consecration. It is painted in grisaille, which was common in that period for the closed panels of altar pieces. Exceptional is that the two figures in color, Peter Scheyfve (standing to the left) and his son (kneeling to the right) are not the main character of the painting, because (obviously) that is Saint Gregory himself (Figure 1). By being the patron of the painting, Peter Scheyfve, who was a rich textile merchant from Antwerp [52], contributed to the transformation of this work by Jheronimus Bosh.

References

1. Heinlen M (1998) An Early Image of a Mass of St. Gregory and Devotion to the Holy Blood at Weingarten Abbey. Gesta 37: 55-62.
2. Landsteiner K, Raubitschek H (1907) Observations on hemolysis and hemagglutination. C Bakt 45: 660.
3. Boyd WC, Shapleigh E (1954) Antigenic relations of blood group antigens as suggested by tests with lectins. J Immunol 73: 226-231.
4. Nowell PC (1960) Phytohemagglutinin: An initiator of mitosis in cultures of normal human leukocytes. Cancer Res 20: 462-466.
5. Reed JC, Druker BJ (2017) Peter C Nowell (1928-2016). Proc Natl Acad Sci USA 114: 4569-4570.
6. Sharon N, Lis H (2007) Lectins. Springer Netherlands ISBN.

7. Sumner JB (1919) The globulins of the jack bean, *Canavalia ensiformis*: preliminary paper. J Biol Chem 37: 137-140.

8. Cavada BS, Osterne VJS, Lossio CF, Pinto-Junior VR, Oliveira MV, et al. (2019) One century of ConA and 40 years of ConBr research: A structural review. Int J Biol Macromol 134: 901-911.

9. Cavada BS, Pinto-Junior VR, Osterne VJ, Nascimento KS (2019) ConA-Like Lectins: High Similarity Proteins as Models to Study Structure/Biological Activities Relationships. Int J Mol Sci 20: 30.

10. Mackler BF, Wolstencroft RA, Dumonde DC (1972) Concanavalin A as an inducer of human lymphocyte mitogenic factor. Nat New Biol 239: 139-142.

11. Greimers R, Trebak M, Moutschen M, Jacobs N, Boniver J (1996) Improved four-color flow cytometry method using fluo-3 and triple immunofluorescence for analysis of intracellular calcium ion ($[Ca^{2+}]_i$) fluxes among mouse lymph node B- and T-lymphocyte subsets. Cytometry 23: 205-217.

12. Griffioen AW, Rijkers GT, Keij J, Zegers BJ (1989) Measurement of cytoplasmic calcium in lymphocytes using flow cytometry. Kinetic studies and single cell analysis. J Immunol Methods 120: 23-27.

13. Farnes P, Barker BE, Brownhill LE, Fanger H (1964) Mitogenic Activity in Phytolacca Americana (Pokeweed). Lancet 2: 1100-1101.

14. Barker BE, Farnes P, LaMarche PH (1966) Peripheral blood plasmacytosis following systemic exposure to Phytolacca americana (pokeweed). Pediatrics 38: 490-493.

15. Bekeredjian-Ding I, Foermer S, Kirschning CJ, Parcina M, Heeg K (2012) Poke weed mitogen requires Toll-like receptor ligands for proliferative activity in human and murine B lymphocytes. PLoS One 7: e29806.

16. Farr SS (2004) Wild Greens. Appalachian Heritage 32: 58-59.

17. Davenport LJ (2012) Tony Joe's Poke Salad. Alabama Heritage; Tuscaloosa Iss 103: 53-54.

18. Raulf M (2019) T Cell: Primary Culture from Peripheral Blood. Methods Mol Biol 2020: 17-31.

19. O'Flynn K, Russul-Saib M, Ando I, Wallace DL, Beverley PC, et al. (1986) Different pathways of human T-cell activation revealed by PHA-P and PHA-M. Immunology 57: 55-60.

20. Tiefenthaler G, Hünig T (1989) The role of CD2/LFA-3 interaction in antigen- and mitogen-induced activation of human T cells. Int Immunol 1: 169-175.

21. Trickett A, Kwan YL (2003) T cell stimulation and expansion using anti-CD3/CD28 beads. J Immunol Methods 275: 251-255.

22. Muul LM, Heine G, SilvinC, James SP, Candotti F, et al. (2011) Measurement of Proliferative Responses of Cultured Lymphocytes. Current Protocols in Immunology 7: Unit7.10.

23. Marrack P, Blackman M, Kushnir E, Kappler J (1990) The toxicity of staphylococcal enterotoxin B in mice is mediated by T cells. J Exp Med 171: 455-464.

24. Leder L, Llera A, Lavoie PM, Lebedeva MI, Li H, et al. (1998) A mutational analysis of the binding of staphylococcal enterotoxins B and C3 to the T cell receptor beta chain and major histocompatibility complex class II. J Exp Med 187: 823-833.

25. Arad G, Levy R, Nasie I, Hillman D, Rotfogel Z, et al. (2011) Binding of superantigen toxins into the CD28 homodimer interface is essential for induction of cytokine genes that mediate lethal shock. PLoS Biol 9: e1001149.

26. Levy R, Rotfogel Z, Hillman D, Popugailo A, Arad G, et al. (2016) Superantigens hyperinduce inflammatory cytokines by enhancing the B7-2/CD28 costimulatory receptor interaction. Proc Natl Acad Sci USA 113: E6437-E446.

27. Faist B, Schlott F, Stemberger C, Dennehy KM, Krackhardt A, et al. (2019) Targeted in-vitro-stimulation reveals highly proliferative multi-virus-specific human central memory T cells as candidates for prophylactic T cell therapy. PLoS One 14: e0223258.

28. Gaundar SS, Blyth E, Clancy L, Simms RM, Ma CK, et al. (2012) In vitro generation of influenza-specific polyfunctional $CD4^+$ T cells suitable for adoptive immunotherapy. Cytotherapy 14: 182-193.

29. Gaucher D, Therrien R, Kettaf N, Angermann BR, Boucher G, et al. (2008) Yellow fever vaccine induces integrated multilineage and polyfunctional immune responses. J Exp Med 205: 3119-3131.

30. Akondy RS, Monson ND, Miller JD, Edupuganti S, Teuwen D, et al. (2009) The yellow fever virus vaccine induces a broad and polyfunctional human memory $CD8^+$ T cell response. J Immunol 183: 7919-7930.

31. Piersma SJ, Leenaars MP, Guzylack-Piriou L, Summerfield A, Hendriksen CF, et al. (2006) An in vitro immune response model to determine tetanus toxoid antigen (vaccine) specific immunogenicity: Selection of sensitive assay criteria. Vaccine 24: 3076-3083.

32. Pichler WJ, Tilch J (2004) The lymphocyte transformation test in the diagnosis of drug hypersensitivity. Allergy 59: 809-820.

33. Monge-Ortega OP, Cabañas R, Fiandor A (2008) Overlap Between DRESS Syndrome and Exanthema Induced by Sulfadiazine in a Patient Treated With Sulfamethoxazole: Utility of the Lymphocyte Transformation Test for Identification of the Culprit Drug. J Investig Allergol Clin Immunol 28: 132-134.

34. Mori F, Fili L, Sarti L, Capone M, Liccioli G, et al. (2020) Sensitivity and specificity of lymphocyte transformation test in children with mild delayed hypersensitivity reactions to beta-lactams. Allergy 75: 2696-2699.

35. Ständer S, Oppel E, Thomas P, Summer B (2017) Evaluation of lymphocyte transformation tests as compared with patch tests in nickel allergy diagnosis. Contact Dermatitis 76: 228-234.

36. Hassan S, Mercuri LG, Miloro M (2020) Does Metal Hypersensitivity Have Relevance in Patients Undergoing TMJ Prosthetic Replacement? J Oral Maxillofac Surg 78(6):908-915.

37. Collinge M, Schneider P, Li D, Parish S, Dumont C, et al. (2020) Cross-company evaluation of the human lymphocyte activation assay. J Immunotoxicol 17: 51-58.

38. Quah BJ, Warren HS, Parish CR (2007) Monitoring lymphocyte proliferation in vitro and in vivo with the intracellular fluorescent dye carboxyfluorescein diacetate succinimidyl ester. Nat Protocols 2: 2049-2056.

39. Quah BJ, Parish CR (2012) New and improved methods for measuring lymphocyte proliferation in vitro and in vivo using CFSE-like fluorescent dyes. J Immunol Methods 379: 1-14.

40. Auguste P, Tsertsvadze A, Pink J, Court R, McCarthy N, et al. (2017) Comparing interferon-gamma release assays with tuberculin skin test for identifying latent tuberculosis infection that progresses to active tuberculosis: systematic review and meta-analysis. BMC Infect Dis 17: 200.

41. Williams J, Maxwell G, Nichola G, Dearman RJ, Kimber I (2014) The lymphocyte transformation test in allergic contact dermatitis: New opportunities. Journal of Immunotoxicology 13: 84-91.

42. Sun X, Kaufman PD (2018) Ki-67: more than a proliferation marker. Chromosoma 127: 175-186.

43. Soares A, Govender L, Hughes J, Mavakla W, de Kock M, et al. (2010) Novel application of Ki67 to quantify antigen-specific in vitro lymphoproliferation. J Immunol Methods 362: 43-50.

44. Popple A, Williams J, Maxwell G, Gellatly N, Dearman RJ, et al. (2016) T lymphocyte dynamics in methylisothiazolinone-allergic patients. Contact Dermatitis 75: 1-13.

45. Nikbakht M, Pakbin B, Brujeni GN (2019) Evaluation of a new lymphocyte proliferation assay based on cyclic voltammetry; an alternative method. Scientific Reports 9: 4503.

46. Sandberg AA, Abe S (1980) Cytogenetic techniques in hematology. Clin Haematology 9: 19-38.

47. Humble JG (1964) The treatment of aplastic anaemia with phytohaemagglutinin. Lancet 1: 1345-1349.

48. Gruenwald H, Taub RN, Wong FM, Kiossoglou KA, Dameshek W (1965) Phytohaemagglutinin in treatment of aplastic anaemia. Lancet 1: 962.

49. Okuda M, Sakaguchi K, Tomiyama S, Takahashi M (1980) Use of phytohemagglutinin in the treatment of maxillary cancer. Arch Otorhinolaryngol 228: 127-134.

50. Mebarki M, Bennaceur A, Bonhomme-Faivre L (2018) Gene to screen: Human-cell-derived organoids as a new ex vivo model for drug assays in oncology. Drug Discovery Today 4: 857-863.

51. Saborowski A, Wolff K, Spielberg S, Beer B, Hartleben B, et al. (2019) In-vivo validation of cancer genes using liver organoids. J Hepatology 68: S671.

52. Ilsink M, Koldewij J, Spronk R (2019) From Bosch's stable: Hieronymus Bosch and the Adoration of the Magi. W Books Zwolle 2019.