

Verisimilitude and Malignancy

A survey of aberrant glycosylation
with regard to blood groups and cancer

Peter D'Adamo

Verisimilitude

Pronunciation: \ver-ə-si-mə-lə-r, -sim-lə-r\

Function: adjective

Etymology: Latin verisimilis

Date: 1681

- 1 : having the appearance of truth
- 2 : depicting realism (as in art or literature)

Some things are just not what they seem..

"Go in peace my daughter.
And remember that,
in a world of ordinary mortals,
you are a Wonder Woman."



Learning objectives I

- ✓ Understand the role of aberrant glycosylation in the malignant process
- ✓ Understand the relationship between glycosylation and lectins
- ✓ Learn about the structure and function of:
 - **Thomsen-Friedenreich (I)** antigen, a 'pancarcinoma' associated antigen
 - **Ligand-Like-Complex (LLC)** an lymphatic 'egress molecule'
 - 'A-like antigen'

Some things are just not what they seem..



▪ **Learning objectives II**

- Distinguish the effects of the ABO, MN and Secretor blood groups with regard to:
 - ✓ The effects of **lectins**
 - ✓ Anti-T antibodies and their induction
 - ✓ 'Horror autotoxicus'
 - ✓ p53 Tumor suppressor

Some things are just not what they seem..



▪ **Learning objectives III**

Learn about modalities which might influence clinical outcomes:

- ✓ Dietary and other inducers of anti-Tn antibodies
- ✓ Dietary lectins with specificity for other aberrant glycoconjugates
- ✓ Dietary and nutraceutical inducers of anti-pancarcinoma antibodies

Glycosyltransferases: The face of cancer

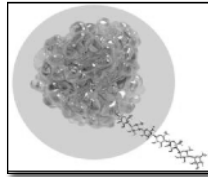
- **Glycomics** is the study of the genomic control of glycosylation
- **Glycosyltransferases** are enzymes that catalyze the transfer of a monosaccharide sugars from one molecule to another
 - **Glucosyltransferases**: transfer glucose
 - **Fucosyltransferases**: transfer fucose
 - **Galactosyltransferases**: transfer galactose
 - **Sialyltransferases**: transfer sialic acid
- Expression patterns of some glycosyltransferase mRNAs are highly regulated in a tissue-specific and developmentally regulated manner
- Others have a widespread so-called “housekeeping” type of distribution

Fats, Proteins and Carbs: A new look

FACTOR INFLUENCING GLYCOSYLATION	
1.	Animal Species
2.	Blood Group Specificity
3.	Age
4.	Site in the Intestines
5.	Position Along the Crypt/ Villus Axis
6.	Diet
7.	Pathology
8.	State of Differentiation and Maturation
9.	Bacterial Status

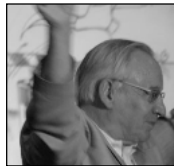
- Glycoconjugates are simply molecules which have a carbohydrate bonded to a fat or protein
 - When bonded to a fat, glycoconjugates are called **glycolipids**
 - When carbohydrates are bonded to proteins, they are called **glycoproteins**
 - When enzymatic produced and reversible, it's a **glycoconjugate**. Otherwise it is a 'advanced glycation end product' (AGE)
- Although some ABO antigens are glycolipids, the vast majority are glycoproteins
- About 11% of the human body is composed of glycoconjugates (glycocalyx)

Aberrant glycosylation: Hallmark of malignancy



- Cell glycosylation depends on the expression and function of various glycosyltransferase and glycosidase systems
- **Malignant transformation is associated with various and complex alterations in the glycosylation process**
 - One of the earliest metabolic defects are alterations to the side chain transferases
 - **Most tumor markers are aberrant glycosylation end-products**
- These changes provide a selective advantage for tumor cells during their progression to more invasive and metastatic forms

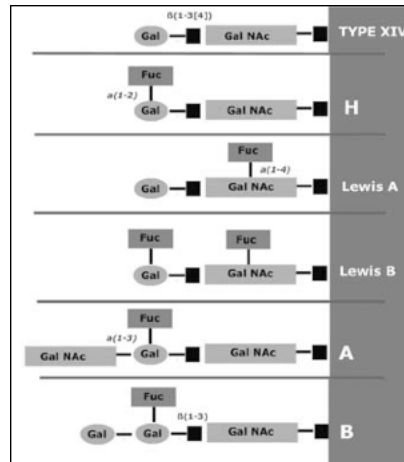
Blood group ‘aberrant glycosylation end-products’



G. Uhlenbruck, MD, Ph.D

- Thomsen-Friedenreich (TFA, T, Tn)
- ‘A-like’ antigens
- ‘Ligand Like Complex’ (LLC)
- Sialyl Lewis X, Sialyl Lewis A
- Ca 19-9, DU-PAN2 (variants)

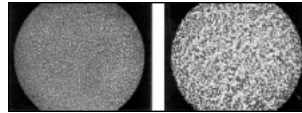
The Blood Group Glycoconjugate Erector Set



Background: 'Secretor Status'

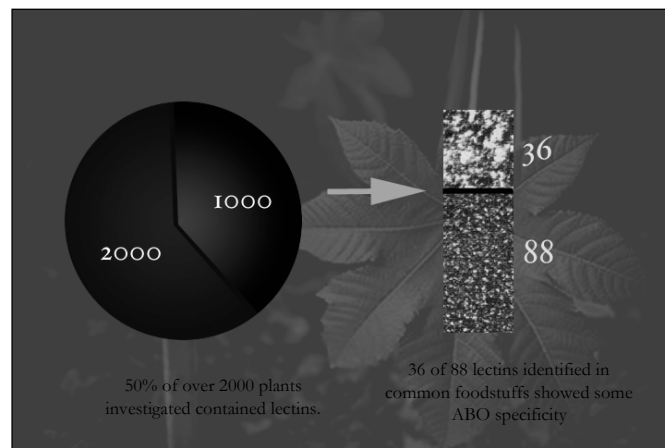
- Some people do and others do not secrete into their saliva antigens corresponding to their ABO blood group
 - Groups A and B secrete their relative antigens, type O secretors secrete H
- Centers around a cluster of genes which control the production of enzymes called 'fucosyltransferases' (FUT)
 - Can test either by saliva or through Lewis blood grouping
 - Also related to CA-19-9, DU-PAN2
- 80-85% of the population are secretors

Backgrounder:
Lectins



- **Lectins** are proteins which specifically bind (or cross link) carbohydrates'
- Name derived from *legere* (to pick or choose). Highly specific!
- The most accepted theory is that plant lectins serve as a sort of primitive immune system, protecting young seeds and beans from parasites and fungi much like our own antibodies protect us
- Diverse class structurally
- 'Two sided molecular Velcro'

Lectins in the world/ lectins in the diet



Joseph Aub, Lectins and Cancer



- In 1963 discovered by chance that there were many surface differences between normal cells and cancer cells.
- Thought 'insane' by colleagues
- Worked with enzymes, attempting to digest certain portions of the cancer cell's surface
- Only one enzyme, derived from wheat germ, showed any effect, agglutinating the cancer cells. When he replaced this enzyme with an identical one from hog pancreas, again, nothing happened
- Found that the wheat germ enzyme was contaminated with a small protein that was responsible for the agglutinating activity
- Aub had discovered a lectin in wheat germ that agglutinated the cancer cells

Background

Thomsen-Friedenreich Antigen

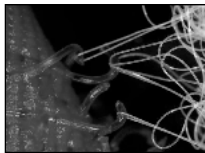
- I. Identified in the 1920's
- II. Also known as:
 - CD176
 - T-antigen
 - TFA
 - Thomsen-Friedenreich oncofetal blood group
- III. Precursor is known as Tn Antigen
 - Formed by incomplete synthesis of mucin-type carbohydrates including MN blood group antigens
 - Structurally similar to group A glycolipids
- IV. Carrier molecule is CD44
 - Multifunctional receptor involved in cell-cell and cell/ extracellular matrix interactions influences adhesion, migration, invasion, and survival

Thomsen-Friedenreich Antigen (T antigen)



- Structure elucidated by G. Uhlenbruck in the 1960's out of work with peanut lectin
- Is 'cryptic' on cell membranes of various normal cells, including epithelial cells, red blood cells, and lymphocytes
- **Pancarcinoma antigen**
- Behaves as an oncofetal antigen in many epithelial tissues and becomes apparent in hyperplasia and malignancy
- Present in colon cancer and inflammatory bowel disease tissue but are absent from normal mucosa
- Provides a link between cancer-associated changes in glycosylation

Thomsen-Friedenreich Antigen (T antigen)



A ligand is a substance that is able to bind to and form a complex with a biomolecule to serve a biological purpose

A galectin is a type of lectin which binds beta-galactoside

- Crucial role in the primary steps of breast and prostate cancer metastases
 - Mediates metastatic adhesion to the endothelium when it recognizes ligands such as galectins or other lectins.
 - Found in the vascular endothelium, liver, bone marrow, and lymph nodes
 - May explain how T-antigen levels relate to carcinoma aggressiveness
 - Increased expression in metastatic tumors
 - **Lectins that bind T-antigen are in some of the common sites of metastatic tumor growth**
 - **Mushrooms!**

Anti-T antigen antibodies (TFA)

RESEARCH ARTICLE | November 4, 2008 | Volume 8, Number 11 | www.nature.com

Inhibition of Spontaneous Breast Cancer Metastasis by Anti-Thomsen-Friedenreich Antigen Monoclonal Antibody JAA-F11¹

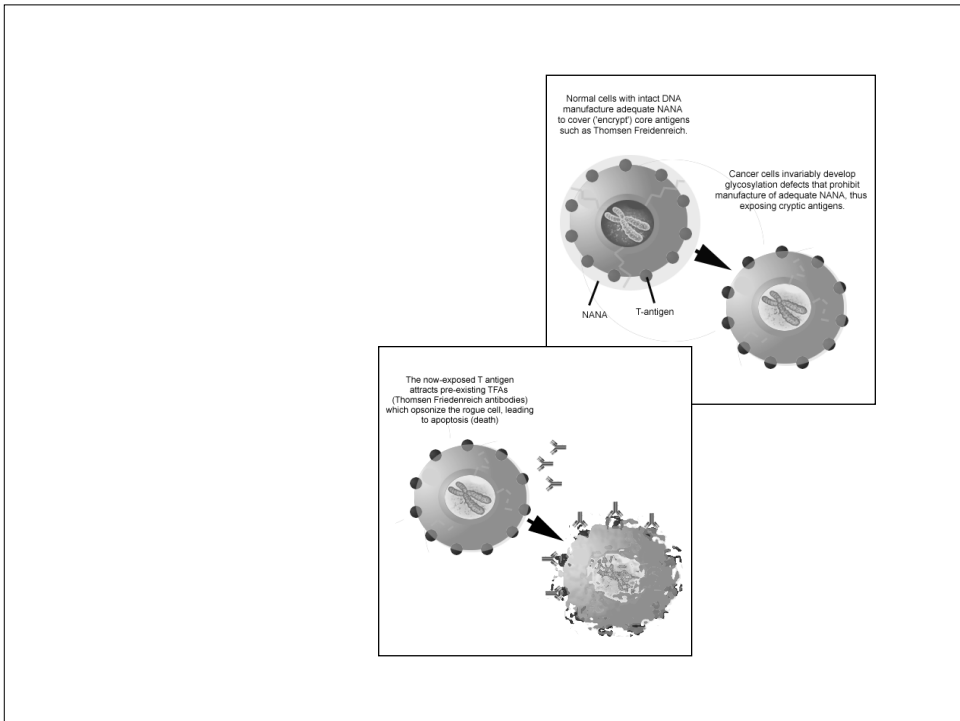
James Hainburg^{1,2}, Jun Yan^{1,2}, Susan Mowbray¹, Olga V. Glinitskaya¹, Virginia H. Huxley¹, Linda Wispf¹, Rene Roy³, Vladimir V. Glinitsky^{1,2,4} and Kate Rittenhouse-Cline^{1*}

¹Department of Microbiology and Immunology, and ²Biotechnical and Clinical Laboratory Sciences, at Buffalo, Buffalo, NY 14214, USA; ³Department of Medical Pharmacology and Physiology, University of Columbia, Columbia, MO 65211, USA; ⁴Department of Pathology and Anatomical Sciences, The University of Illinois at Chicago, Chicago, IL 60607, USA; ⁵Department of Chemistry, University of Quebec at Montreal, Montreal, Quebec, H3C 3P8, Canada; ⁶Department of Biochemistry, University of Missouri, Columbia, MO 65211, USA; ⁷Hôpital Maisonneuve-Rosemont, Montreal, Quebec, H3T 1M5, Canada

Abstract
Thomsen-Friedenreich antigen (TF-Ag) is expressed in many carcinomas, including those of the breast, colon, bladder, and prostate. TF-Ag is important in adhesion and metastasis, and as a potential immunotherapy target. We hypothesized that passive transfer of JAA-F11, an anti-TF-Ag monoclonal antibody, may create a

is the Thomsen-Friedenreich antigen (TF-Ag) encoded by Thomsen, Friedenreich, and the H5051 (1). TF-Ag is a disaccharide glycosylated in proteins by an N-glycosyltransferase. It is cryptic or only found on normal cells. (citing reference only, not found)

- All humans normally possess antibodies that react with the Thomsen-Friedenreich antigens
- These anti-Thomsen-Friedenreich antibodies (TFA) are typically IgM class antibodies
- Lowest titers in blood group A
- The most common induction of TFA is through the gut
 - Many gram negative organisms induce TFA
 - Some show blood type preference in terms of BGA degradation



Neo-Springerism (anti-Tn therapy)

- George Springer was the main clinical researcher of T and Tn antigens. Lifelong blood group researcher, especially regarding blood type substances in natural products
- **Better differentiated cancers (least aggressive) produce more T antigen while the least differentiated cancers (most aggressive) produce the greatest amount of Tn**
- Springer's vaccine consisted of three parts:
 - Chemically degraded O-group blood cells (providing T and Tn antigens)
 - Salmonella typhi vaccine or typhoid vaccine (which contains T and Tn antigens)
 - Calcium phosphate (he believed the T and Tn antigens could stick to this)
- In my practice, we employ the standard typhoid vaccine to this effect, as it in itself constituted 1/2 of the Springer anti-Tn vaccine therapy.

Results at time of Springer's Death

<i>Five year survival</i>		
stage II	7 patients	100 percent alive with no evidence of disease (N.E.D.)
stage III	6 patients	100 percent alive, 3 with N.E.D., 3 with cancer but fully functional
stage IV	6 patients	100 percent alive; 4 with N.E.D., 2 with cancer but fully functional

<i>Ten year survival</i>		
stage II	5 patients	100 percent alive
stage III	5 patients	60 percent alive
stage IV	4 patients	75 percent alive

Springer GF, Desai P, Tegtmeier H, Carlstedt SC, and Scanlon EF (1994). T/Tn antigen vaccine is effective and safe in preventing recurrence of advanced human breast carcinoma. *Cancer Biother* 9, 7 - 15.

Backgrounder:
‘A-likeness’

- I. Some cancers contain an A-like substance even when they occur in persons who are not A or AB.
 - In both normal and neoplastic tissue all persons, there are blood group A-like antigens present which are usually inaccessible to the immune system
- II. In the course of the immune response to a growing cancer, the antigen becomes accessible.
 - Then an A person, who cannot make anti-A, will be more likely than an O person to tolerate the cancer
- III. Antibodies against Tn antigen cross-reacted with A glycolipids, since Tn antigen and A glycolipids share terminal GalNAc
 - **Therefore, Tn antigen was also concluded to be an A-like antigen in a broad sense**

Why are cancers ‘A-like’?

There are increasing reports of BGAs [e.g., Le(x) (an isomer of Le(a)), Le(y) (an isomer of Le(b)), T, Tn, "A-like"] appearing as "new" antigens on malignant tissue. Their presence and membrane density appears to correlate with the metastatic potential of the tumor. This often parallels loss of normal BGAs (e.g., ABH) from the tissue.

Garratty G. Immunol Invest
1995 Jan-Feb;24(1-2):213-32

- Incomplete synthesis of carbohydrate chains (e.g. loss of ABO antigens)
- Accumulation of precursor carbohydrates (e.g. accumulation of I antigen which is one of the precursors of ABO)
- Synthesis of new carbohydrates (e.g. expression of A-like antigens in cancer of O & B hosts)
- Many monoclonal antibodies raised against cancer cells have been shown to react with blood group carbohydrates

Background:
‘Ligand Like Complex’



- Altered glycosylation predicts lymph node involvement in breast cancer
- 373 primary breast cancers, in a 24 year retrospective study, which were stained for the binding of Helix pomatia (escargot snail) lectin (HPA)
- This lectin is nominally specific for GalNAc
- Strong association between HPA binding and the presence of lymph node metastases.
- This binding molecule, an obscure ligand-like complex (LLC) , is apparently absent from normal and early (non-metastatic) breast cancer cells
 - As the malignancy worsens and LLC is increasingly elaborated, the tumor cells become paradoxically more susceptible to agglutination by HPA
 - Snails are mentioned as a cure for breast cancer in 15th century Italian monographs.

Coming and Going: BGAs in Malignancy

Tissue, organ	Normal appearance of BGAs	In Malignancy
Colon	Present	Absent
Bladder	Absent	Present
Prostate	Present	Absent
Liver	Absent	Present
Squamous	Present	Absent
Endometrium	Absent	Present
Stomach	Present	Absent
Thyroid	Absent	Present
Esophagus	Present	Absent

“Deletion or reduction of blood group A or B antigen in tumors of A or B individuals is clearly correlated with the degree of malignancy and metastatic potential.”
Int J Cancer 1998 Apr 13;76(2):284-9

Blood Groups, Malignancy and Diathesis



- AE Mourant (*Blood Groups and Disease*):
 - The most striking associations are with cancers, nearly all of which are associated with group A, as are clotting diseases
 - While bleeding diseases, mostly due to a deficient clotting mechanism, are, on the contrary, associated with group O
 - Other diseases, which appear to be associated with group O are the auto-immune diseases.
 - The contrast with the cancer-group A association is an interesting one in view of the suggestion of MacFarland Burnett that there is a fundamental antithesis between the two classes of disease.



Backgrounder:
'Horror Autoxicus'

- **Horror autoxicus** essentially implies that the immune system is inherently disinclined to attack tissues that contain antigenic similarities to our own
- This was originally postulated by Paul Erlich at the turn of the century to explain the specificities of auto-immunity
- Blood-group-A cancer patients had the greatest and uniform suppression of the level of TFA agglutinins, irrespective of age, cancer stage or tumor morphology, and lower levels of anti-B isohemagglutinins

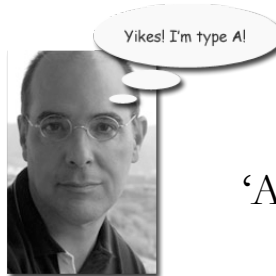
Secretor status and tumor markers



A hapten is a small molecule which can elicit an immune response only when attached to a large carrier.

Selectins are a family of cell adhesion molecules (or CAMs)

- CA 19-9 is a hapten of human sialyl-Lewis(a)
 - Ligand for selectins and promote the metastatic process by facilitating interaction with the endothelium of distant organs
 - Adenocarcinomas of the pancreas and stomach
 - Most colon and gall bladder tumors,
 - Half of primary and metastatic ovarian tumors.
- The secretor transferase (FUT2) influences the amount of circulating Ca 19-9 by elongating the structure to Lewis(b)
 - Because of this secretors have smaller amounts of circulating Ca19-9 and non-secretors have much larger amounts
 - Lewis Double Negative (LDN) have ZERO



‘A Proneness’

- Horror autotoxicus (LLC, A-like)
- Lower anti-Tn antibodies
- Viscosity issue when stressed
- P53 (epigenetic silencing)
- P-glycoprotein (multiple drug resistance)
- More angiogenesis prone
- MM, Secretor subtypes most prone
- Propensity is nice but the question is really survivorship



Modalities
Dietary

- Rich in anti-cancer lectins; low in other lectins
 - Soy, Lima beans, Peanuts, Amaranth, Mushrooms
- Blood group specific
 - System sparing
 - Anti-adhesive for cancer lectins
- Polyamine sequestering
 - Diminish growth factors
- Rich in side chain cleaving enzymes (r-glycosidases)
 - Coffee
- Epigenetic effects
 - p53, re-methylation, histone acetylation

I'll have the escargot.



- Lectin produced by the albumin gland (reproductive tract) acts to protect the eggs from the attack of microorganisms
- Considered a blood type A specific lectin, but typically will only agglutinate A cells under special circumstances
- More predictably it binds "aberrant N-acetylgalactosamine (GalNAc) glycoconjugates."
- Helix pomatia agglutinin (HPA) agglutinates *S. typhi*
- Resists digestion pretty well

Ooohh! It comes with a side of mushrooms.



- Agaricus lectin stimulates an undifferentiated colon cancer cell line to differentiate into gland like structures
- The adhesion molecule epCAM is involved in this
- VFA (broad bean lectin) also does it
- “A. bisporus lectin is a reversible non-cytotoxic inhibitor of epithelial cell proliferation which deserves study as a potential agent for cancer therapy.” (*Gut* 1999 May;44(5):709-14)
- This is probably related to its specificity for the alpha-NacGal epitope on T an TN antigens.

And for desert.... Jackfruit!



- A well-ripe fruit emits a very pleasant smell, has a sweet taste and the flesh is waxy and golden-yellow in color
- ‘Juicy Fruit’ gum was fashioned after the flavor of Jack Fruit
- The seeds are as big as quail egg, which are often boiled, fried or roasted with salt
- Major lectin in the seeds and pulp is Jacalin (JAC)
- JAC binds Thomsen-Friedenreich. Its TF binding inhibits several glycoproteins elaborated by endometrial tissue undergoing disease processes
- JAC produced dose-dependent and non-cytotoxic inhibition of proliferation in human colon cancer cells



Modalities

Anti-Tn inducer serologicals

- Fernand Widal (1862-1929) found that blood serum from a typhoid carrier caused a culture of typhoid bacteria to clump, whereas serum from a typhoid-free person did not
- Typhoid vaccine
 - Springerism
 - Oral or injectible
 - Give *Arctium* (burdock) spp (*Arctiin* lignans)
 - Calcium (Tricalcium Phosphate)
 - Drink 2-4 cups of coffee one hour before
- Pneumococcus vaccine in the old days

Salvia species, Tn and CAMs



- Many colon cancer lines are agglutinated by lectin-producing *Salvia* species (Tn specific lectins) (*Eur J Biochem* 2000 Mar;267(5):1434-40)
- *Salvia miltiorrhiza* inhibits Neutrophil-Endothelial Adhesion
- Salvianolic acid B attenuates VCAM-1 and ICAM-1 expression in TNF-alpha-treated human aortic endothelial cells. (*Jpn J Pharmacol.* 2002 Nov;90(3):276-80.)

Baptisia tinctoralis, Marrubium vulgare



- *Baptosins, Quinolizidine, Baptisine, Cystisine (alkaloids)*
- *Baptisin (bitter glucoside)*
- *Baptin (purgative glucoside)*

- *Baptisia* (wild indigo) is known to possess Tn like antigenic attributes (*Glycoconj J 1995 Feb;12(1):55-62*)
- *Baptisia tinctoralis* raises anti-typhoid agglutinins
 - “*Baptisia tinctoralis* in low dilutions produces a form of antibodies to the bacillus typhosus, i.e. the agglutinins. Thus it raises the natural bodily resistance to the invasion of the bacillary intoxication, which produces the typhoid syndrome.” (*Boericke, Pocket Materia Medica*)
- *Marrubium* (horehound) species are also known to induce anti-Tn, due to the possessing of anti-Tn specific lectins. All *Marrubium* species tested seem to have anti-Tn lectins



Strange..
Not my normal binding site

Conclusions

- Rational and evidence based
- Food as medicine
- Plays well with allopathy
- Treats the patient, then the pathology
- Complements all other approaches
- Cost effective and easy to do long-term
- This is one approach among many. The greater the number of novel approaches towards the cancer dilemma, the greater the chances that they will summate and produce the exceptional outcome



Closing Thought

“If life was fair, Elvis would be alive
and all the impersonators would be dead.”

Johnny Carson

Resources

Books

- The Complete Blood Type Encyclopedia (D'Adamo)
- Cancer: Fight it with the Blood Type Diet (D'Adamo)
- The GenoType Diet (D'Adamo)
- Lectins and Glycoconjugates in Oncology (Springer-Verlag)
- Essentials of Glycobiology (pubmed onlinebook)

Web

- Main Website
 - www.dadamo.com
- The Individualist wiki:
 - <http://www.dadamo.com/wiki/wiki.pl/Welcome>
- Lectin database
 - <http://www.dadamo.com/lecster2/Lecster.htm>

Audio-Video

- NAP Professional Services
 - <http://www.northamericanpharmaceutical.com/professional/>

Training

- Institute for Human Individuality (IFHI)
 - <http://www.dadamo.com/ifhi/index.htm>