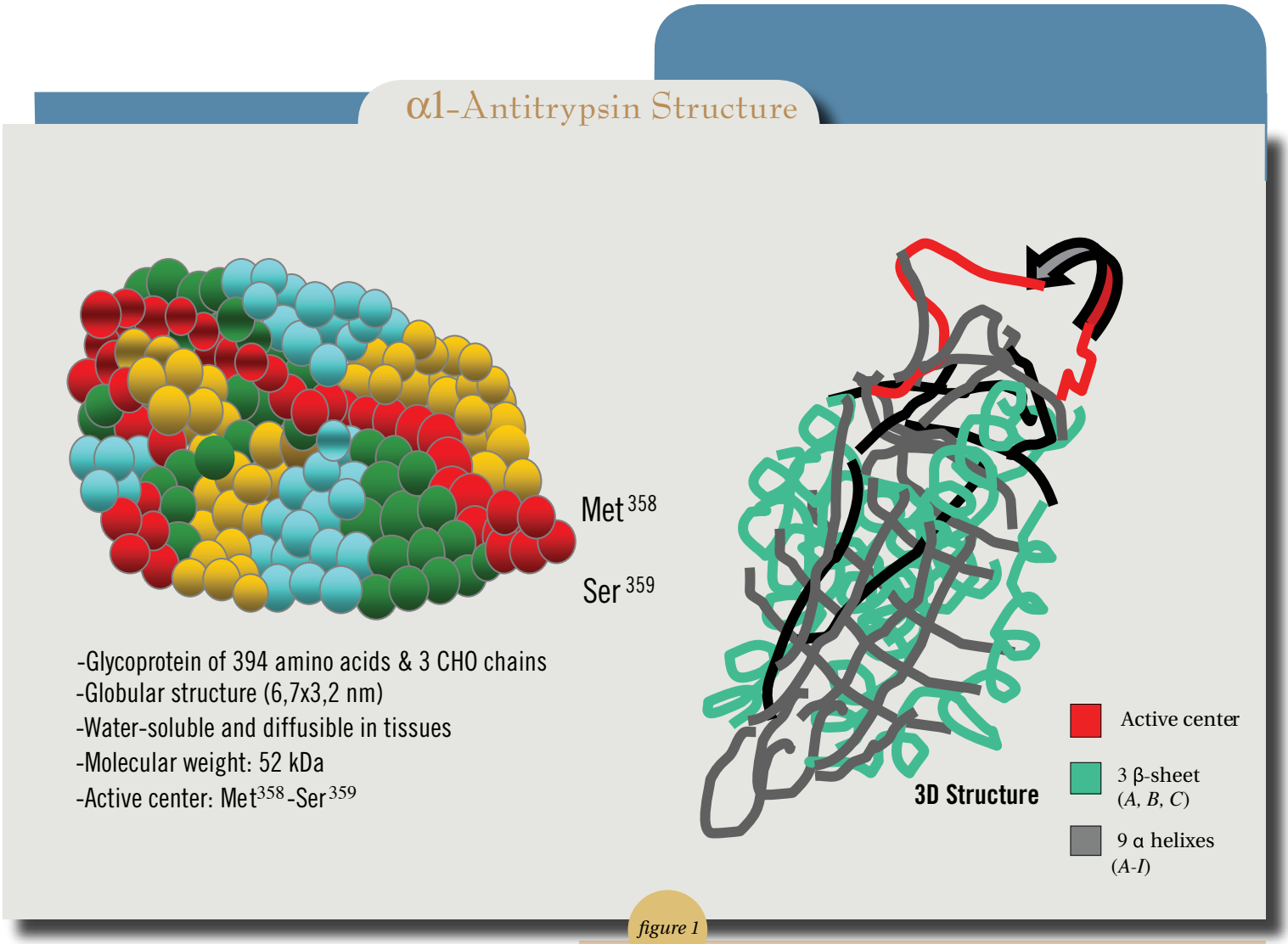

figure 1

α1-antitrypsin (α1-AT) structure. α1-AT is a globular glucoprotein, constituted by a central chain of 394 aminoacides (red) with an active site of Methionine and Serine, and 3 laterally-attached carbohydrate chains (blue, yellow and green). The X-ray crystal structure of AAT shows 3 β-sheets and 9 α-helixes supporting the active site (red) of the protein.

α1-Antitrypsin and FM

By Ignacio Blanco, MD

α1-Antitrypsin is a powerful anti-inflammatory protein, very abundant in blood and tissues.

AS THE ARCHETYPAL MEMBER OF THE SERPIN (Serine Protease Inhibitor) super family—a very large family of approximately 500 structurally-related proteins–α1-Antitrypsin typically inhibits most serine proteases (i.e. enzymes breaking peptide chains in proteins, essential to several physiological processes, including blood clotting, immunity, inflammation, and digestion) when their quantity is excessive in one's body and their activity begins to negatively affect one. Serine proteases include elastase, trypsin, tryptase, etc. (figs.1, 2 and 3).

Apart from this well-known anti-serine protease capacity, α1-Antitrypsin protects from microorganism infections, and contributes to inhibition of some bacterial toxins (endotoxins) and cytokines (i.e. transmitters allowing cell-to-cell communication) favoring inflammation. Moreover, α1-Antitrypsin helps to decrease vascular injury in inflammatory diseases by limiting the uncontrolled activation of cells in the endothelium (i.e. the thin layer covering blood vessels); increases the production of anti-inflammatory cytokine IL-10; modulates cell activity (activation and apoptosis); protects proteins from oxidative damage; and stimulates tissue repair when damaged (fig. 4*).

Although several cells synthesize α1-Antitrypsin, the liver is the primary source of this protein (fig. 5*). The α1-Antitrypsin gene is located in the long arm of chro-

α1-Antitrypsin Deficiency was discovered only 40 years ago in patients suffering from pulmonary emphysema and liver cirrhosis.

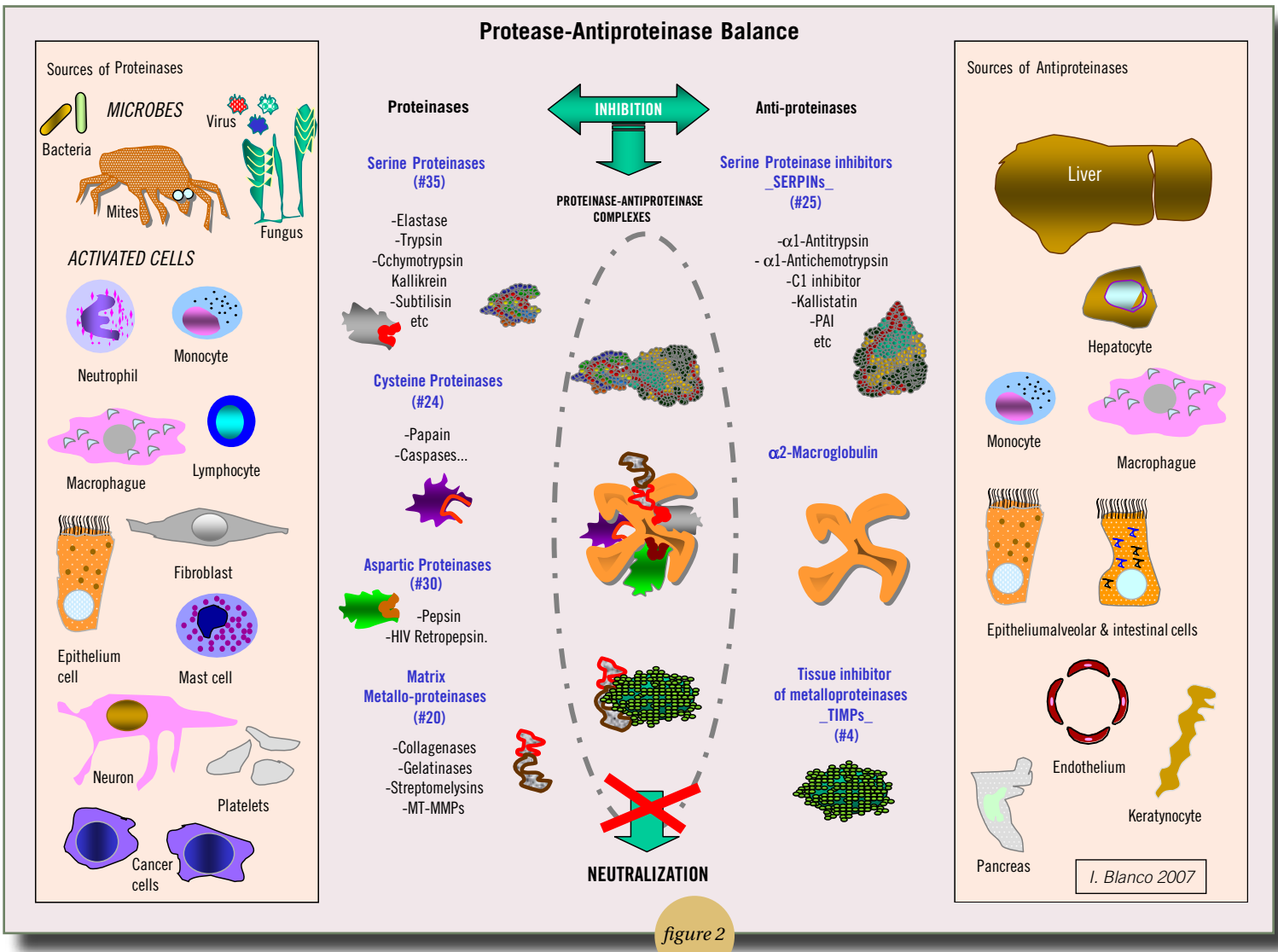

figure 2

The adjusted balance between proteases and antiproteases in the human body. Proteases liberated by microorganisms or activated cells are neutralized by antiproteases to avoid its proteolytic effect damaging tissues. α1-AT is the archetypical representative of the serpin family. Other relevant antiproteases are α2-macroglobuline (a wide-spectrum anti-panprotease) and TIMPs (Tissue Metalloproteases Inhibitors).

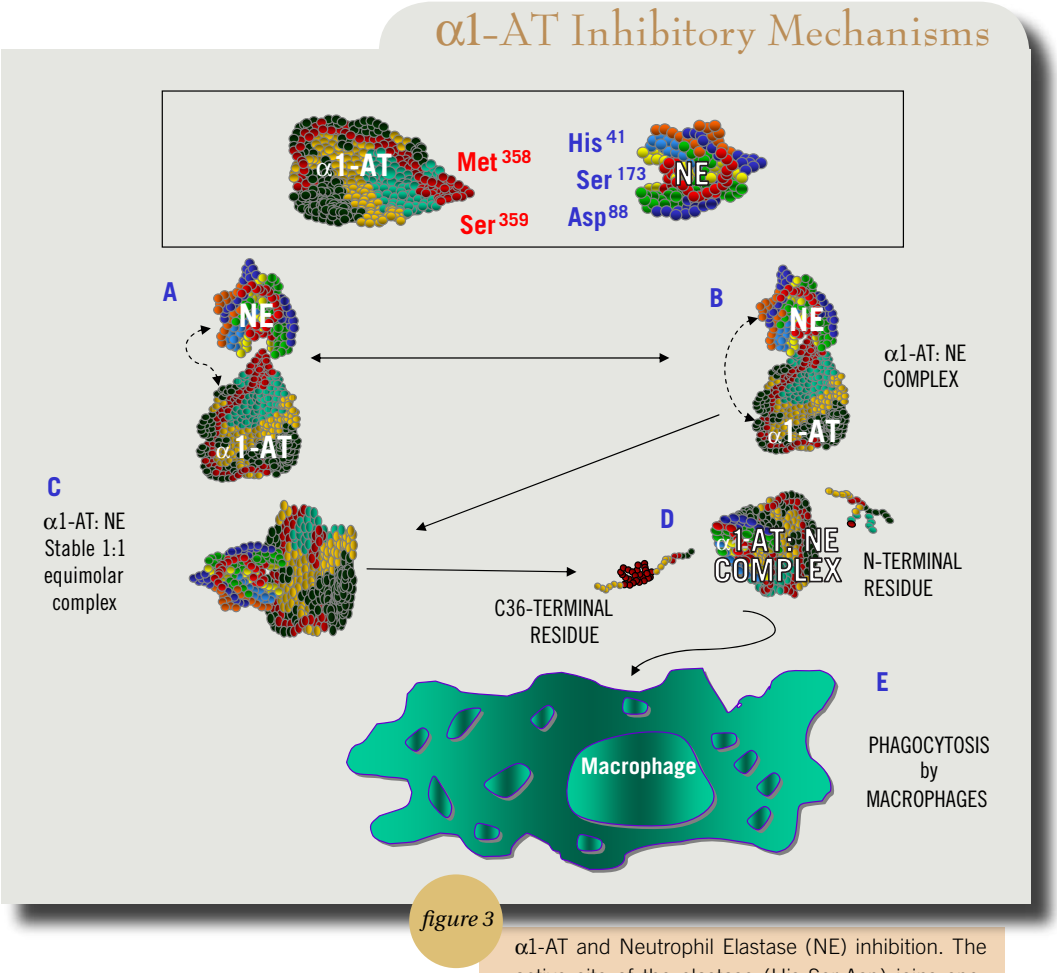

figure 3

α1-AT and Neutrophil Elastase (NE) inhibition. The active site of the elastase (His-Ser-Asp) joins specifically to the active site (Met-Ser) of α1-AT. α1-AT inhibits serine proteases by forming covalent (1:1) complexes, leading to an irreversible (suicide) inhibition of both molecules. Inactivated complexes are rapidly cleared by macrophages.

mosome 14 and has two alleles, one from each parent. Alleles are designated by alphabet letters. Normal alleles are called M and are present in more than 80 percent of normal individuals. Thus, a normal individual has an MM phenotype. The most common abnormal alleles are called S and Z.

α1-Antitrypsin Deficiency is a hereditary condition, passed from parents to their children through their defective genes. The most common phenotypes found in daily practice are five combinations of M, S and Z alleles, namely MM, MS, MZ, SZ, and ZZ. Serum levels for these phenotypes are: MM: 100 percent (range 80-120 percent); MS: 80 percent (range 75-85 percent); SS: 60 percent (45-70 percent); MZ: 60 percent (50-70 percent); SZ: 40 percent (30-45 percent); and ZZ: 15 percent (10-20 percent) (fig. 6). In very rare circumstances individuals can inherit null alleles, characterized by the total absence of serum α1-Antitrypsin. At least 20 other variants affect either the amount or the function of the α1-Antitrypsin molecule, but in clinical practice most α1-Antitrypsin Deficiency-related diseases are linked to the Z allele. Serum levels less than 35 percent (that is, 50 mg/dL or 11 μM) are associated with an increased risk for pulmonary emphysema. Cut-off values for risk for other α1-Antitrypsin Deficiency-related diseases (including fibromyalgia) have not been established yet.

IN RECENT YEARS OUR TEAM HAS REPORTED A RELATIONSHIP BETWEEN α1-ANTITRYPSIN AND FM.

S and Z proteins are abnormal proteins that fail to fold properly and tend to polymerize, being retained in liver cells (hepatocytes). Thus, 90 percent of Z and 40-50 percent of S proteins are retained in the cytoplasm of the hepatocytes, where they are normally degraded by the ubiquitin-proteasome system (fig. 7*). Liver deposits and decreased secretion of α1-Antitrypsin to the blood can result in liver diseases in infants, children, teenagers, and adults (neonatal hepatitis, hepatic cirrhosis, and hepatocarcinoma) and serious lung diseases in adults (Chronic Obstructive Pulmonary Disease - COPD). α1-Antitrypsin Deficiency was discovered only 40 years ago in patients suffering from pulmonary emphysema and liver cirrhosis. Consequently, most research has been focused on lung and liver diseases. Eventually, other inflammatory diseases—such as vasculitis of the Wegener type and relapsing panniculitis—were added to the list of diseases related to α1-Antitrypsin Deficiency, as well as several other inflammatory and neoplastic diseases, such as bronchial asthma, bronchiectasis, rheumatoid arthritis, intracranial and abdominal aneurysms, arterial dissections, inflammatory bowel disease, psoriasis, chronic urticaria, mesangiocapillary glomerulonephritis, multiple sclerosis, pancreatitis, several neoplasms and, lastly, FM.

α1-Antitrypsin Deficiency is one of the most common serious hereditary disorders in the world. Although it can affect individuals in all racial subgroups worldwide, it is much more common in Caucasians of Northern European heritage.

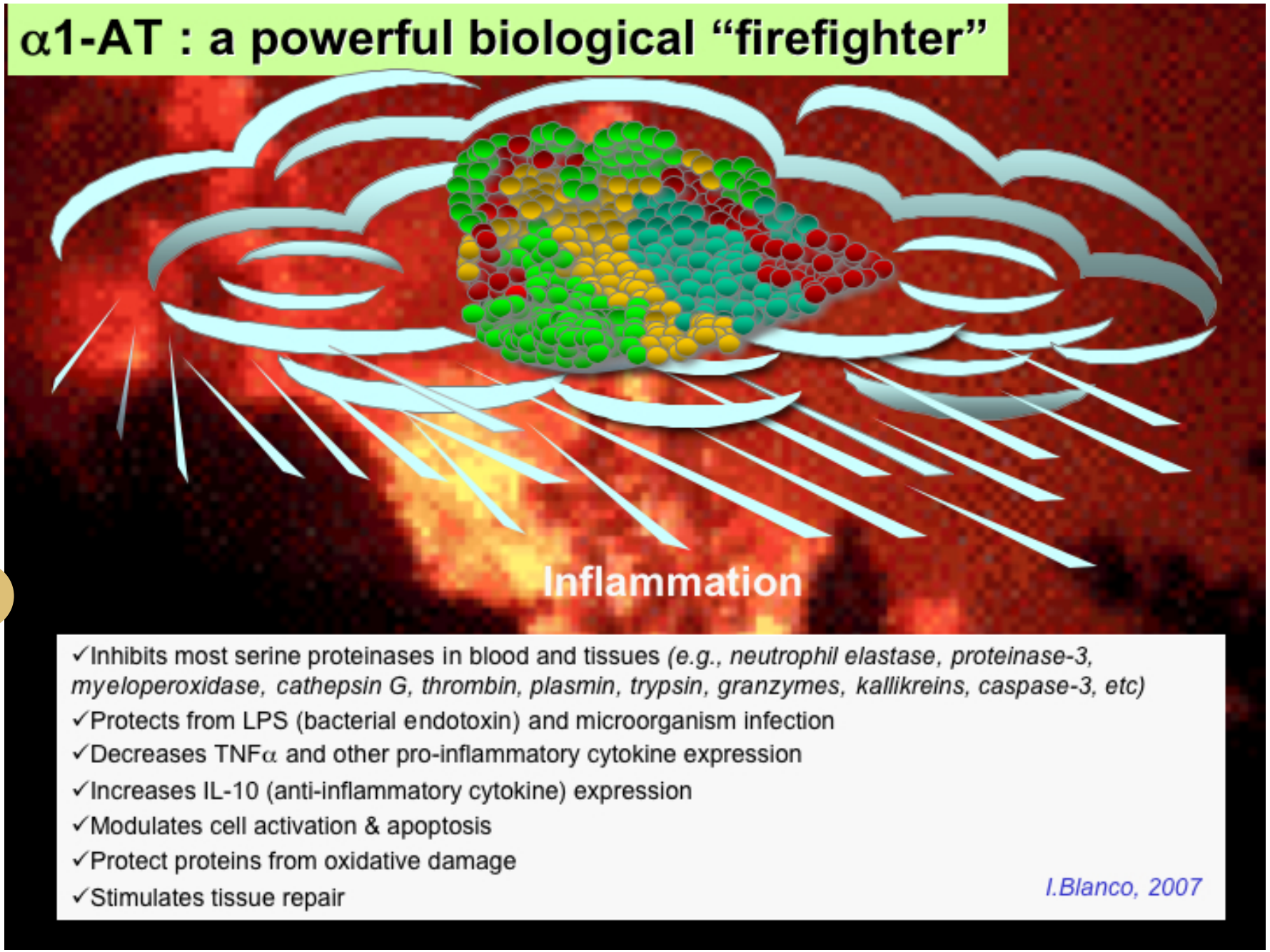

figure 4

α1-AT properties. In the figure are summarized several major functions of α1-AT protein in the human body.

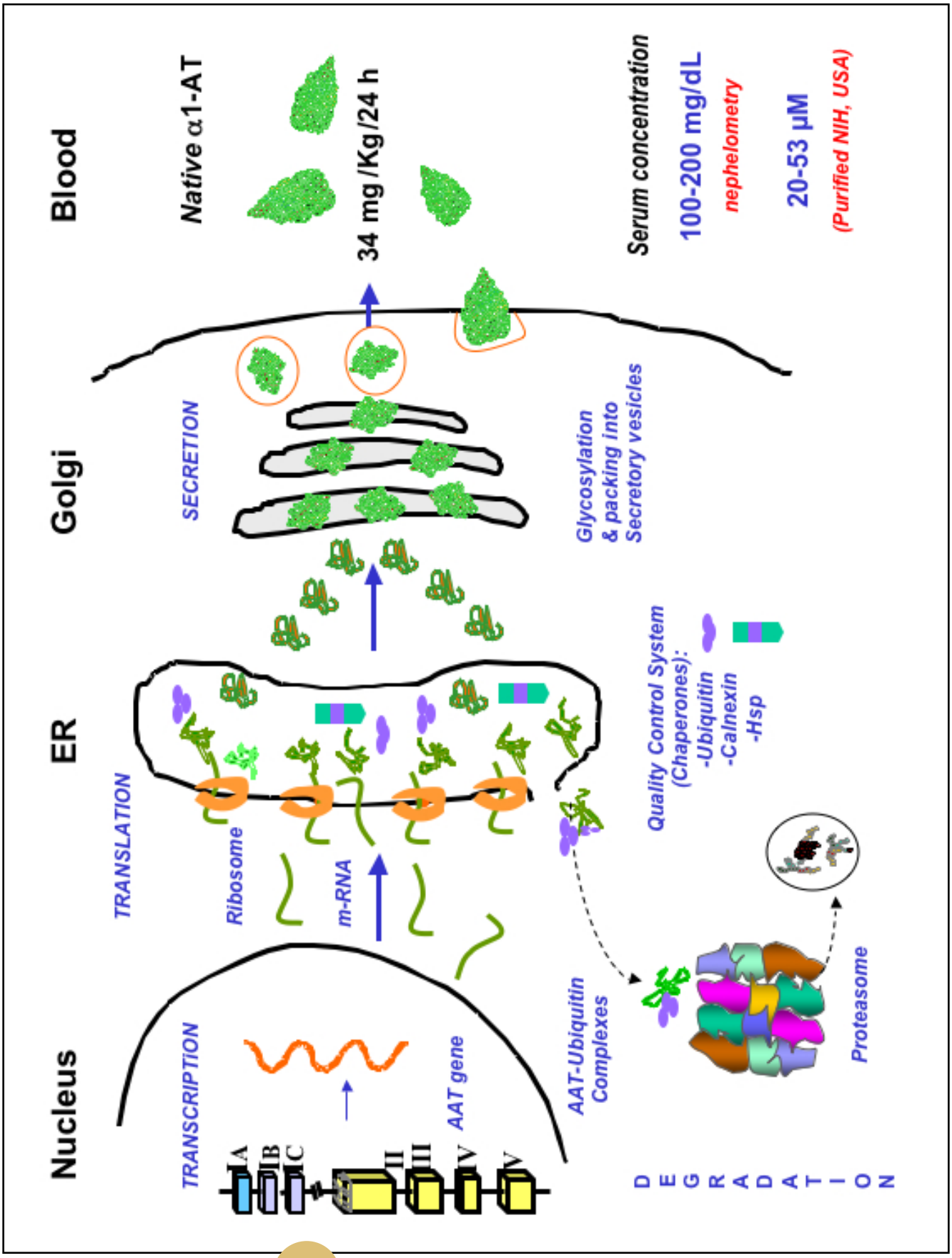

figure 4

Liver cells (hepatocytes) as the main manufacturing site of αα1-AT . Normally, a messenger molecule (mRNA) is created by copying α1-AT gene. Ribosomes (orange structures within Endoplasmic Reticulum, ER) build α1-AT. Normal α1-AT (M) folds with the help of chaperones (i.e. ubiquitin, calnexin, heat shock protein), moves to the Golgi apparatus into secretory vesicles, where it is secreted into blood. A few α1-AT molecules fold incorrectly, being delivered into the proteasome for degradation.

Thus, the average prevalence in the United States (one out of 11 individuals) is higher in white Americans and practically nonexistent in Asian Americans, with the following gradient: White Americans > Hispanic Americans > Mexican Americans > Black Americans > Asian Americans. Published estimates indicate that, of the 5,354,089 FM patients in the U.S., a total of 478,681 (8.9 percent) suffer from α1-Antitrypsin Deficiency (fig. 8).

TREATMENT

In 1987, the U.S. Food and Drug Administration (FDA) approved the use of purified human α1-Antitrypsin products derived from human plasma for augmentation therapy in patients with pulmonary emphysema related to severe α1-Antitrypsin deficiency (phenotypes ZZ and null). This augmentation therapy raises the levels of α1-Antitrypsin in blood and tissue, protecting the lungs from continuous damage and destruction by proteases. Therapeutic concentrates are prepared from blood plasma of blood donors, being administered intravenously at a dose of 60 mg/kg once a week, or 120 mg/kg every two weeks, or 180 mg/kg every three weeks.

Available studies indicate a lowered overall mortality, a slower rate of pulmonary function decline, and a reduction of the incidence of lung infections in patients with α1-Antitrypsin Deficiency-related emphysema. Augmentation therapy is considered safe and effective in patients with α1-Antitrypsin Deficiency and necrotizing panniculitis. In addition, a ZZ case with persisting multi-organ vasculitis has been reported to respond dramatically to the administration of purified α1-Antitrypsin.

Lastly, several observations indicate that augmentation therapy has been effective in controlling long-term FM symptoms in two female sisters with the ZZ phenotype

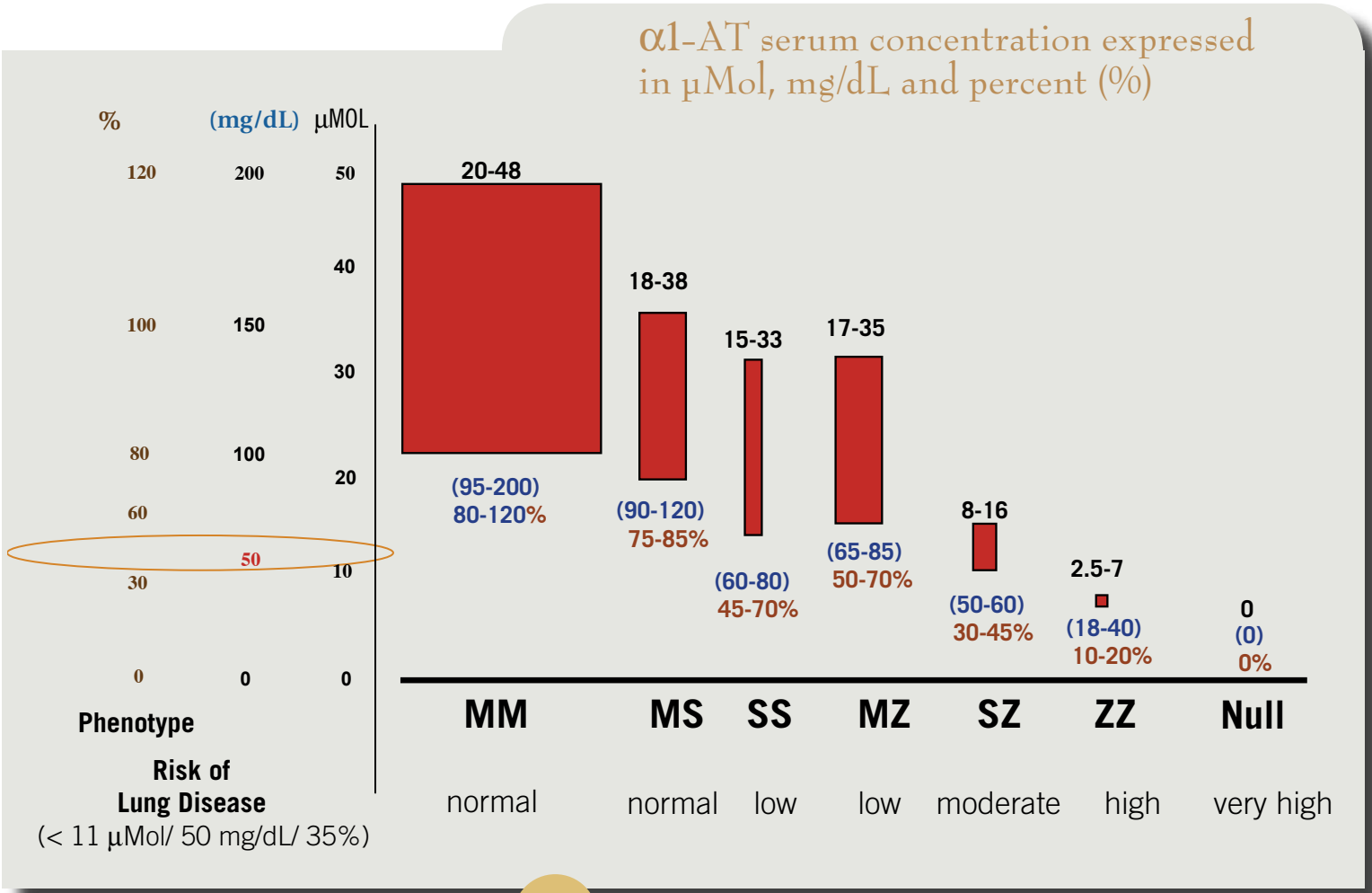

figure 6

α1-AT serum concentrations from different phenotypes expressed in percent (%) of the expected values, mg/dL (measured by nephelometry) and the purified standard (µM) used in the US Registry. Bar size is proportional to each phenotype prevalence.

and in another, third MZ patient. Adverse reactions to α1-Antitrypsin infusions are rare, and no viral transmission has been observed to date. The major problem with purified human α1-Antitrypsin therapy is its high cost and the relative scarcity of the product. There are several ongoing projects to produce synthetic α1-Antitrypsin by using genetic engineering techniques, and ongoing advances in the field of gene therapy have been reported as well.

> SEVERAL OBSERVATIONS INDICATE THAT **AUGMENTATION THERAPY** HAS BEEN **EFFECTIVE** IN **CONTROLLING LONG-TERM FM SYMPTOMS.**

α1-ANTITRYPSIN DEFICIENCY AND FM

In recent years our team has reported a relationship between α1-Antitrypsin and FM. This report has arisen from the basis of a number of clinical, epidemiological, and pathological evidences, which can be summarized as follows:

A clinical alert. In the early 1990s, two α1-Antitrypsin deficient ZZ Spanish sisters with severe FM started augmentation therapy for respiratory problems. Surprisingly, after two to three α1-Antitrypsin augmentation therapy infusions, the women's FM symptoms gradually disappeared. Both did well during the next years. But in 1998, there was a worldwide α1-Antitrypsin augmentation therapy shortage, which forced Spanish patients to stop α1-Antitrypsin augmentation therapy infusions for four to five consecutive months every year. The sisters' FM symptoms slowly recurred when infusions were stopped, but disappeared every time infusions were resumed. Both sisters have continued under our care for the past 15 years, with similar results.

A favorable response to α1-Antitrypsin augmentation therapy in an FM patient with moderate α1-Antitrypsin Deficiency, and muscle and endothelium deposits of α1-Antitrypsin in muscle biopsy samples. Three years ago, our team studied a second intriguing case: a patient with severe FM also diagnosed with bronchial asthma and moderate α1-Antitrypsin Deficiency. In 2004, this patient accepted a clinical trial with α1-Antitrypsin augmentation therapy. The effect of augmentation therapy on the control of pain was striking. FM symptoms relapsed with placebo, and improved again with treatment. A quadriceps muscle biopsy showed large deposits of amorphous material in myofibrils (filaments forming muscle fibers), which reacted positively to the α1-Antitrypsin antibody. Moreover, α1-Antitrypsin deposits were found in small vessels of muscles. Given the favorable clinical response to the treatment, the patient continued with the α1-Antitrypsin augmentation therapy, and two recent sequential control muscle biopsies showed that α1-Antitrypsin deposits disappeared together with the patient's widespread pain (unpublished observation).

An epidemiological study carried out in Asturias (Northern Spain) between 2003 and 2005 showed that severe α1-Antitrypsin Deficiency was found twice as often in FM patients than in the general population, suggesting that α1-Antitrypsin Deficiency might play an important role in FM development and clinical expression in, at least, a subset of FM patients.

A pilot study on muscle biopsies performed on a series of 23 FM patients (13 with and 10 without inherited α1-Antitrypsin Deficiency) found diffuse muscle capillary and myofibril deposits of non-polymerized α1-Antitrypsin in α1-Antitrypsin Deficiency patients, in contrast to eight non-FM controls. Bigger α1-Antitrypsin Deficiency was related to bigger α1-Antitrypsin deposits. These findings suggested that the presence of α1-Antitrypsin accumulations in soft body tissues could be the consequence of an exaggerated local expression of this protein, secreted by endothelium, myocites, and extracellular matrix-resident cells in response to proteases, cytokines, free radicals, etc., to ameliorate tissue damage, and that α1-Antitrypsin Deficiency could play an important role in the development of FM, probably related to low α1-Antitrypsin concentrations in tissues and the loss of α1-Antitrypsin efficacy.

These observations should open novel perspectives for research, diagnosis, and treatment of FM. The positive clinical response to α1-Antitrypsin augmentation therapy in some patients should suggest that in FM related to α1-Antitrypsin Deficiency, basal low tissue α1-Antitrypsin concentrations would be a predisposing factor for FM development and symptom expression. Due to the high prevalence of α1-Antitrypsin Deficiency in FM and the possible efficacy of α1-Antitrypsin augmentation therapy to control FM symptoms, investigation of α1-Antitrypsin Deficiency, starting with the determination of α1-Antitrypsin serum levels and electrofocusing for phenotype characterization if indicated, should be recommended to FM patients.

RESEARCH

In recent years a novel mechanism of hyperalgesia—induced by Protease-Activated Receptors 2 (PAR2) expressed in nociceptive afferent neurons—has been delineated, pointing to a direct role of PAR2 and proteases in pain transmission. Interestingly, hyperalgesia induced by PAR2 could even be elicited at subinflammatory stimuli concentrations. PAR2 are extensively distributed throughout the skin, the central and peripheral nervous system, the vascular system, the digestive tract, and other organs and systems of the human body. More recently, it was demonstrated that several serine proteases (such as trypsin from neurons and epithelial cells; tryptase from mast cells; granzymes A, elastase and protease-3 from neutrophils; human tissue kallikreins from injured tissues; and proteases from bacteria, virus, and mites) can signal cells by cleavage/activation of PARs. Interestingly, gene expression of some of these proteases is modulated by sex steroid hormones. It is important to highlight that activated serine proteases are tightly controlled by endogenous inhibitors, mainly Serpins (including α1-Antitrypsin) and α2-macroglobulin (fig. 9*).

α1-Antitrypsin Deficiency can offer a

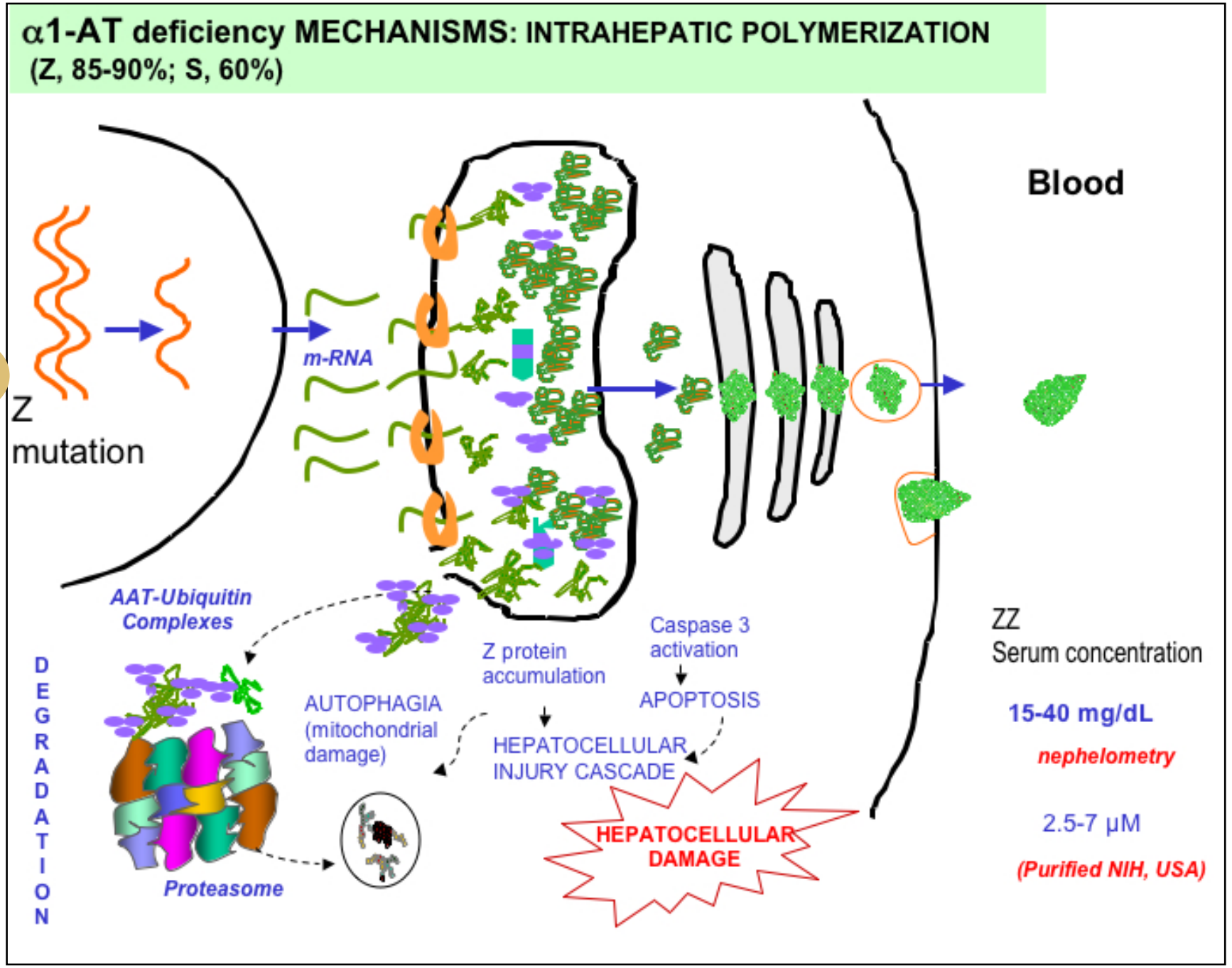

figure 7

In α1-AT Deficiency, 85-90 of Z and 60% of S proteins folds incorrectly. These mis-folded molecules tend to link in chains, called polymers. The accumulation of polymers expands the ER, reduces blood secretion and increases proteasome degradation. Protein accumulation (specially Z protein) also induces autophagy, mitochondrial injury, cell apoptosis and hepatocyte injury, which can cause liver cell damage.

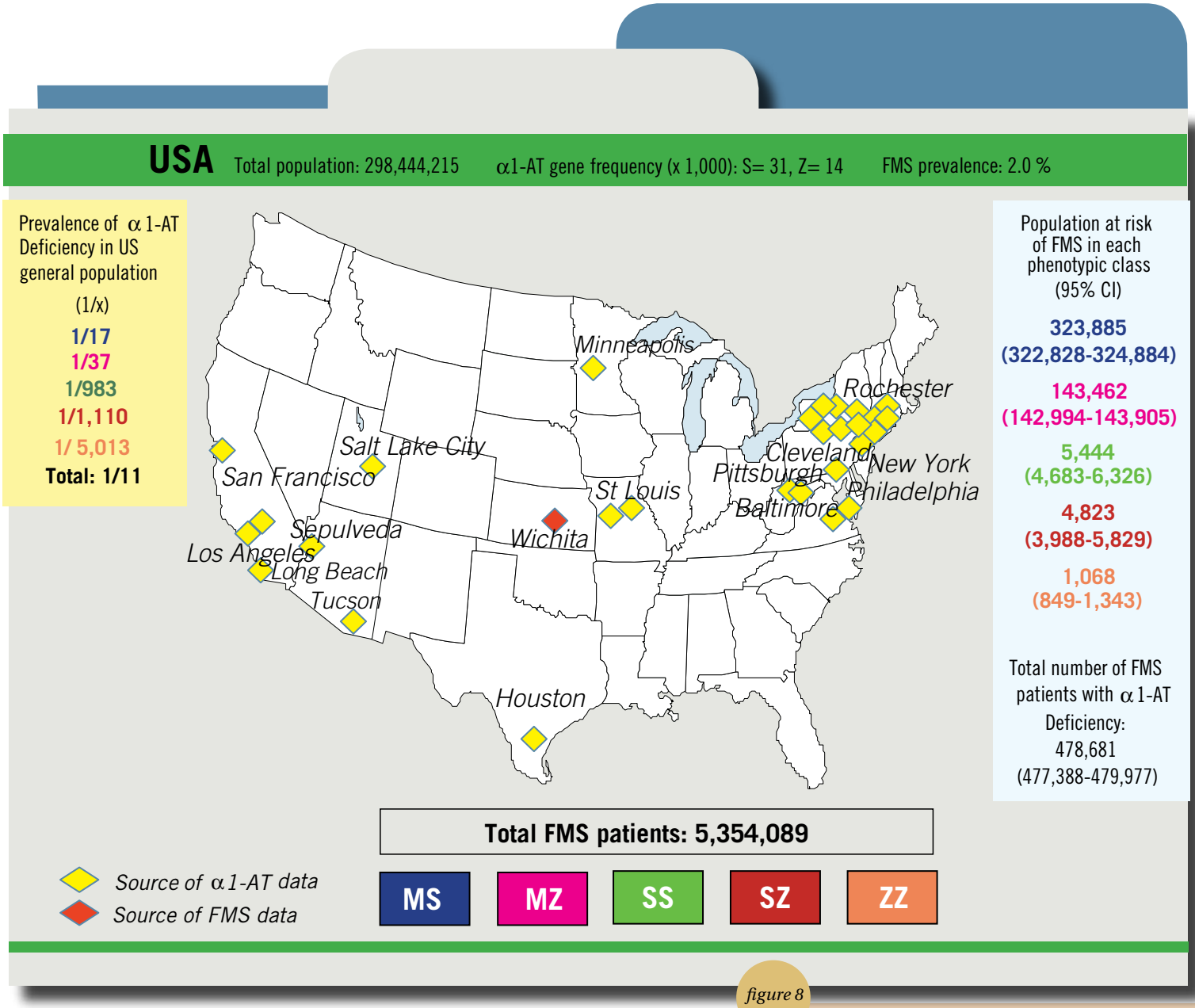

figure 8

Estimates of α1-AT gene frequency, prevalence and numbers of α1-AT deficiency individuals with FM in USA. Calculations are based on available epidemiological peer-reviewed published data (Ref: I. Blanco et al: *JM Pain* 2007).

valuable model to study a suspected protease-antiprotease imbalance in FM. Therefore, we are using samples from normal and α1-Antitrypsin deficient subjects to investigate serine proteases and PAR2 expression patterns in the skin, subcutaneous tissue, and striated muscle to identify a PAR2 pain pathway activated by proteases, not being neutralized by α1-Antitrypsin. If the activation of a PARs pathway was demonstrated in FM, this finding would explain several intriguing issues, such as: (1) the pain mechanisms in FM patients; (2) why FM is mostly a disorder of females (explained because the sex hormone-dependent expression of some serine proteases); and (3) why α1-Antitrypsin is able to control FM symptoms.

The author would like to acknowledge the editorial assistance of Ms. Jimena Blanco (Licenciée en Traduction, Université de Genève, Switzerland).

Dr. Ignacio Blanco works as a clinician specializing in both Internal and Respiratory Medicine, as well as a researcher in the field of α1-Antitrypsin and FM, at the Hospital Valle del Nalón in Asturias, Northern Spain.

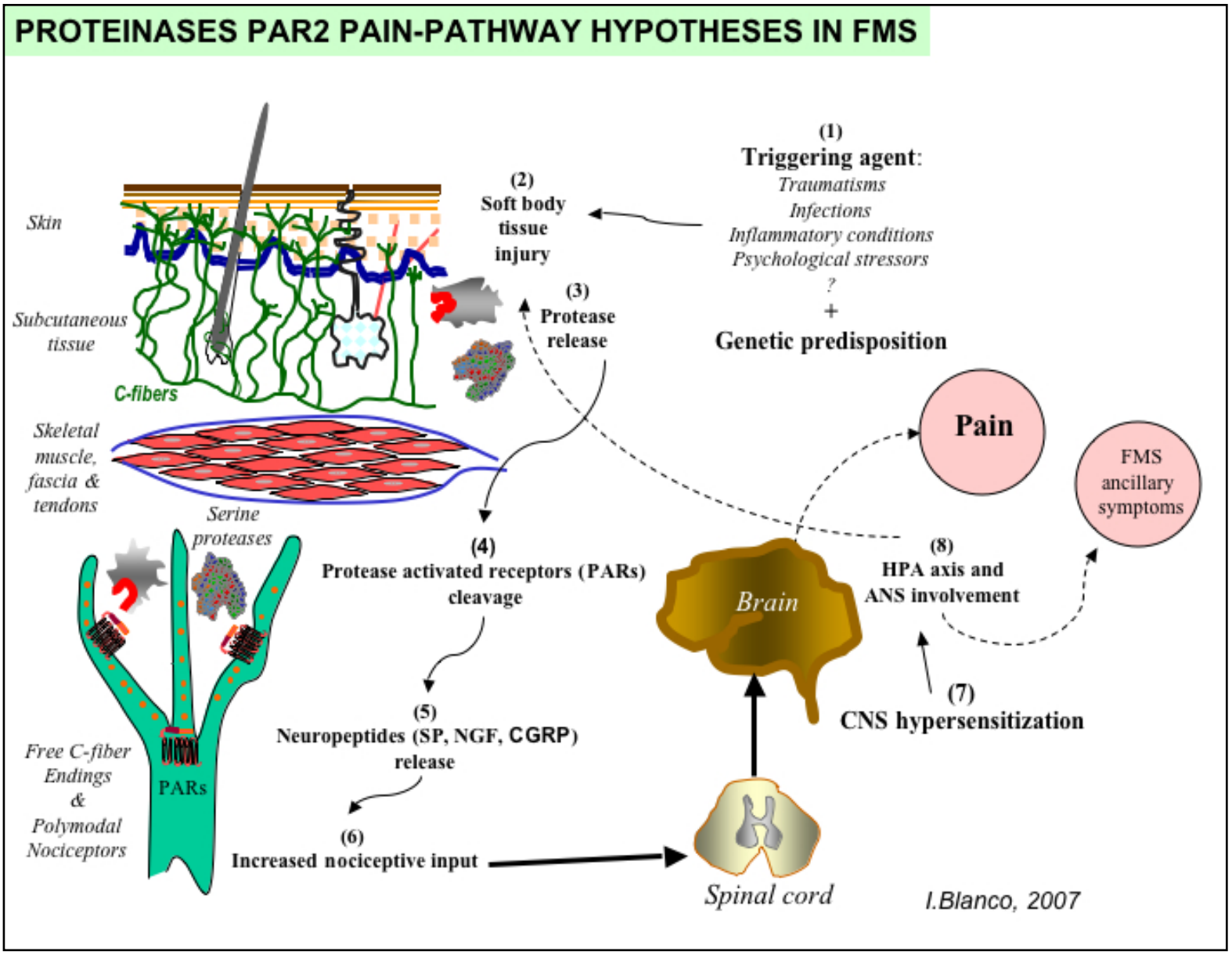

figure 7

FMS could result from the abnormal imbalance between inflammatory and anti-inflammatory substances. Should the burden of inflammatory mediators overcome anti-inflammatory biological defenses (either by an excessive production of the former products or by a dismissed amount of anti-inflammatory proteins), an abnormal imbalance could be generated, causing a low-grade persistent stimuli for subcutaneous nociceptors and provoking, in its turn, central sensitisation. Biological tissue repair mechanisms should be also important in the long-term evolution of FMS. Proteinase Activated Receptors type 2 (PAR2) have been involved in nociception, and can be activated for the local excess of serine proteases (even in sub-inflammatory concentrations).