Kuwait Pharmacy Bulletin
DRUG INFORMATION FOR THE HEALTH PROFESSIONAL
Vol 20 No 1 Spring 2016 ISSN 1028-0480

Toll-like receptors and allergic disease suppression: is there a link ?

Introduction

Asthma and allergic rhinitis (AR) are two common respiratory diseases that represent a major health care issue affecting large populations of all ages. The pathogenesis of asthma involves several inflammatory cells such as T-helper 2 lymphocytes (Th2), macrophages, mast cells, eosinophils, and neutrophils.

As shown in Figure 1, the inflammatory process starts when an allergen/antigen or any other provoking agent enters the respiratory airways. Antigen presenting cells (APCs) such as dendritic cells (DC), will aid in presenting or preparing the allergen to the immune system. Th2 cells recognise the antigen, differentiate, and begin expressing various inflammatory cytokines which include, interleukin (IL)- 4, 5 and 13 (1). The induced cytokines further stimulate B-lymphocytes for antibody production. Once B-cells undergo differentiation, they release IgE antibodies that are specific to the presented allergen. Upon second exposure of the allergen in the airways, IgE antibodies will move and bind to the Fc region on the surface of mast cells causing them to burst and release various mediators including histamine, prostaglandins and leukotrienes. The inflammatory mediators will eventually promote airways obstruction and mucus hypersecretion.

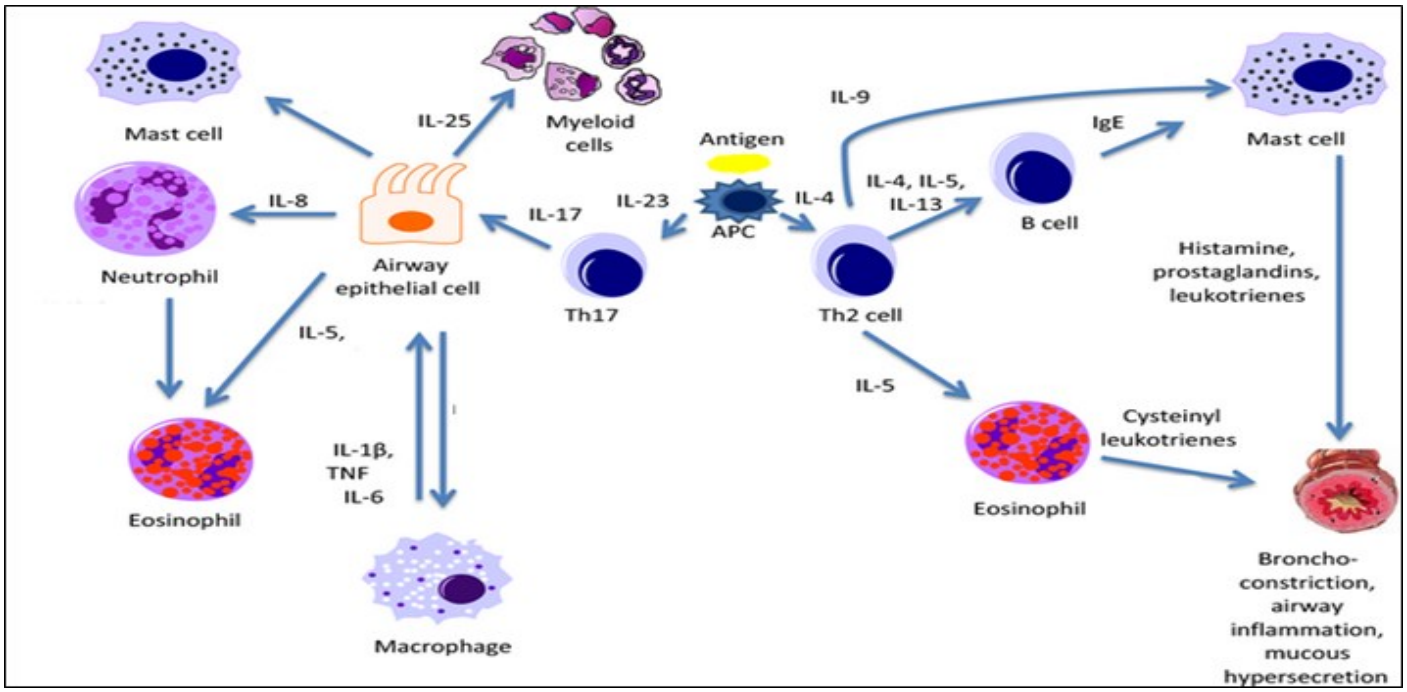

Figure 1. Pathophysiology of asthma. APC: antigen presenting cell, Th17: T helper 17, IL: interleukin, IgE: immunoglobulin E (Adapted from ref 2).

AR is a disease of the upper respiratory airways and is characterised by nasal pruritus, sneezing, rhinorrhea and congestion. Approximately 400 million individuals suffer from AR worldwide, with high-

In this issue

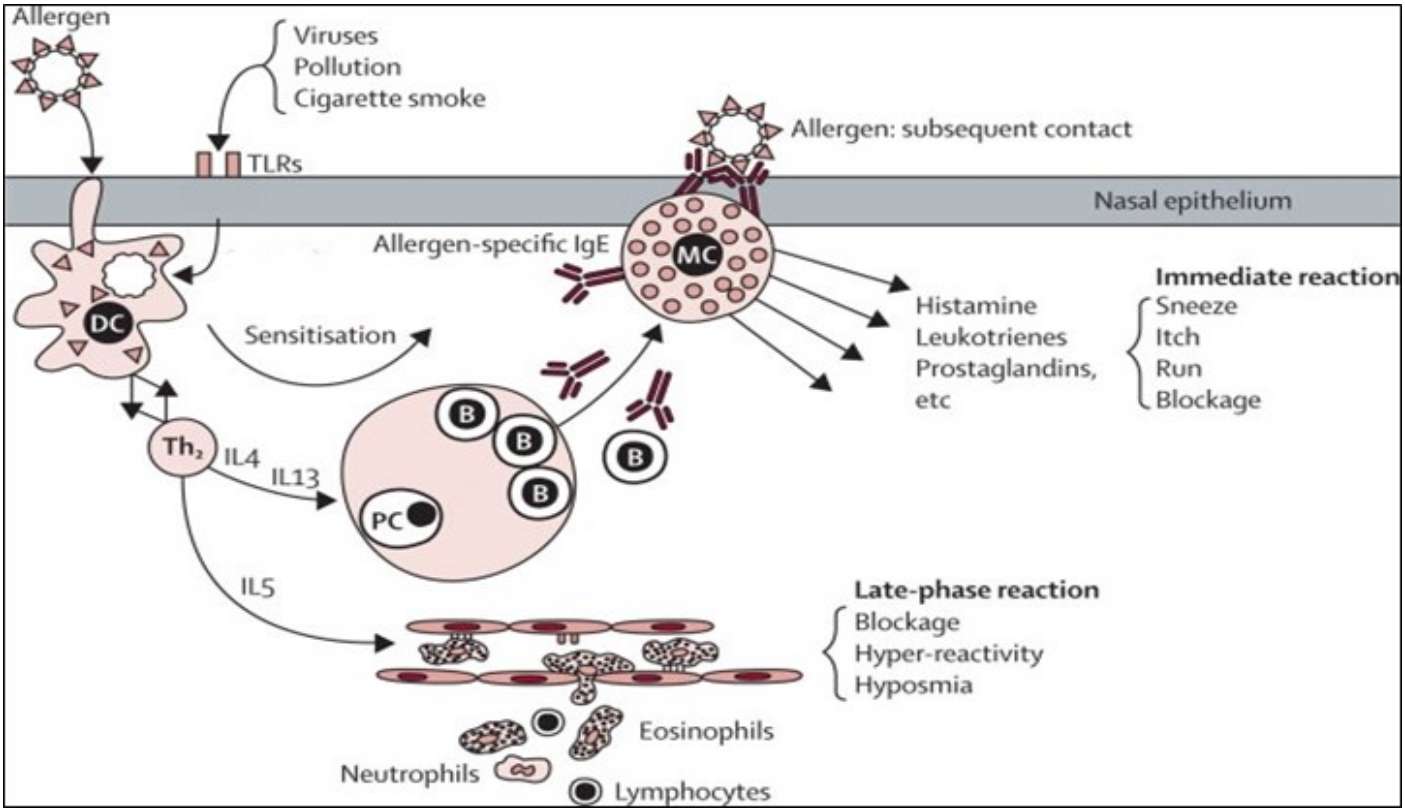

Figure 2. Pathophysiology of allergic rhinitis DC: dendritic cell PC: plasma cell B: basophil MC: mast cell Th2: T helper 2 cell IL: interleukin (Adapted from ref 4).

est prevalence in industrial nations (2).

Closely related to the pathophysiology of asthma, the inflammatory process in AR starts when the allergen presents on the surface of nasal mucosa. As shown in Figure 2, the antigen is presented by DC to Th2 cells, which in turn induce expression of IL-4, 5, 10, and 13 (3). Further differentiation of plasma cells into B-cells will produce antigen specific IgE antibodies. Upon subsequent contact of the antigen with the nasal epithelial layers, IgE in sensitised individuals will bind to the Fc region on the surface of mast cells and basophils. As a consequence of that interaction, histamine and other inflammatory mediators will result on an immediate reaction manifested as sneezing, itching and runny nose. Further activation of other immune cells such as: neutrophils and eosinophils will direct a late-phase reaction (Figure 2) that leads to nasal blockage, hyper-reactivity, and hyposmia,

Unmet medical needs

The main current available treatment options for asthma are inhaled corticosteroids (ICS), long and short acting selective B_2 agonists, long and short acting anti-muscarinic bronchodilators, leukotrienes receptor antagonists and phosphodiesterase type 4 inhibitors. Among these drug classes, ICS demonstrate the highest efficacy and are considered first line treatment options for almost all patients newly diagnosed with asthma (3). However, 10% of asthmatics are said to be steroid-resistant or have refractory asthma, which is attributed to the increased levels of Th17 and its related cytokine IL-17.

Regarding AR, the available treatment options can be divided into oral or topical drugs. The topical treatments are corticosteroids, antihistamines, cromones, anticholinergics and decongestants, whereas the oral treatments are antihistamines, corticosteroids, antileukotrienes, and decongestants. Topical corticosteroids need several days to show an effect, while the patient is actively suffering from the symptoms. Topical decongestants can lead to rhinitis medicamentosa if overused. Oral antihistamines cause sedation as the main problem for many patients.

Therefore the socioeconomic burdens and problems arising from the current treatment approaches (refractory asthma and uncontrolled AR) are unmet needs that require new therapeutic drugs.

Table 1. Summary of TLR types, location and ligands

TLR	Location	Ligands
TLR2/1	Cell-surface	Triacyl lipoproteins, peptidoglycan
TLR2/6	Cell-surface	Diacyl lipoproteins, zymosan
TLR3	Endosomes	dsRNA
TLR4	Cell-surface	Lipopolysaccarides
TLR5	Cell-surface	Bacterial flagellin
TLR7	Endosomes	ssRNA
TLR8	Endosomes	ssRNA
TLR9	Endosomes	Unmethylated CpG DNA
TLR10	EndosomeP	Profilin-like protein

Toll-like receptors

Toll-like receptors (TLRs) are a group of cellular receptors that are known to be the sensors in detecting microbes, foreign particles, and products of viruses, bacteria, fungi and protozoa. In mammals, including humans, TLRs constitute the first line host defense against infections in the innate immune system and help in the propagation of the adaptive immune response. The expression of TLR is greatly increased on cells that are part of the innate immune system such as macrophages, neutrophils, DCs and mast cells. In addition, they are found expressed in T and B lymphocytes and non-immune cells. The main function of TLRs is to recognise pathogen associated molecular patterns (PAMPs). Once TLRs recognise PAMPs that are unique to microbes, an inflammatory response will be initiated, leading to the production of several cytokines such as IL-6 and 12 and TNF-α.

There are 10 and 13 identified members of TLRs in humans and mice respectively and are located either on the cell surface or are intracellular/ endosomal. Cell surface TLRs (TLR1, TLR2, TLR4, TLR5, and TLR6) have the ability to detect microbial components that are near the plasma membrane. In contrast, TLRs that are located in the endosomes (TLR3, TLR7, TLR8, and TLR9) are capable of sensing foreign nucleic acids found within the cell.

Types of TLRs and their ligands

Each TLR can recognise and detect different types or collections of microbe-derived products (Table 1). TLR2 in conjugation with TLR1 or TLR6 recognises lipoproteins that are products of gram-positive bacteria. In addition, TLR2 can detect peptidoglycan and zymosan (fungi). TLR3 can detect double-stranded RNA (dsRNA) produced from single-stranded RNA (ssRNA) viruses such as: West Nile virus (WNV), respiratory syncytial virus (RSV), and encephalomyocarditis virus during their replication phase. Lipopolysaccharide (LPS), which is the main constituent of gram-negative bacterial cell wall, can activate TLR4, whereas bacterial flagellin can activate TLR5. Moreover, TLR7 and TLR8 can both recognise ssRNA from several viruses. TLR9 detects unmethylated CpG (cytidine-phosphate-quannosine) bacterial DNA and TLR10 detects profilin-like protein.

Structure of TLRs

TLRs are members of a large family known as Toll-interlukin1 receptors (TIR). All members share a structural similarity in their intracellular/ cytoplasmic domain. Both TLR and IL-1 have the same intracellular region that is called TIR domain; however they differ in their ectodomain region. The extracellular domain of TLR that binds to PAMPs contains 16-28 leucine-rich repeats (LRR), each of which possess 24-29 amino acids with a conserved sequence of XLXXLXLXX. The LRRs form a horseshoe-like structure consisting of a β-strand and α-helix connected by loops. This horseshoe-like shape increases the surface area required for the interaction between the receptor and the ligand.

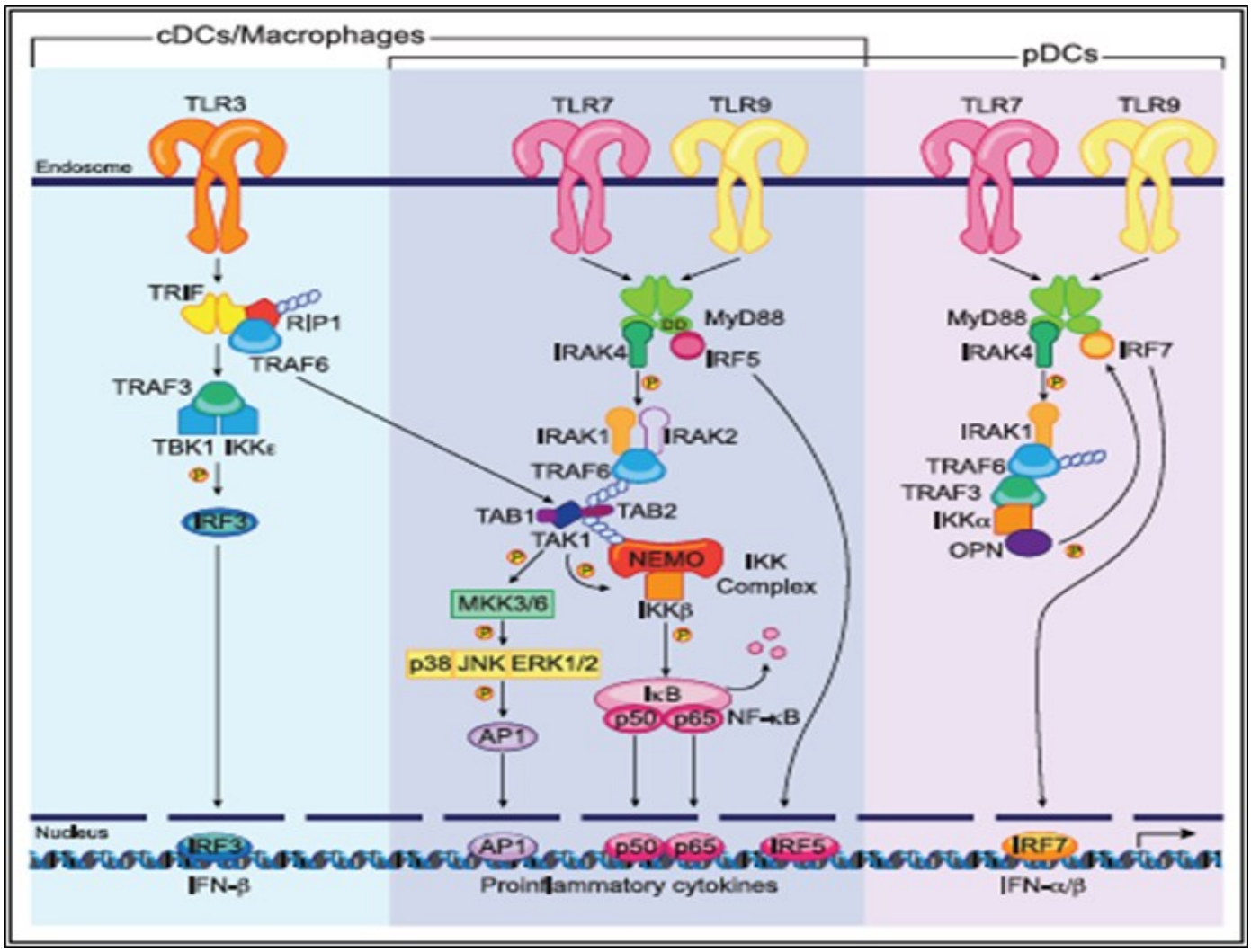

Figure 3. Signaling pathways by TLRs: MyD88: myeloid differentiation factor 88, DD: death domain, IRAK4: IL-1R associated kinase 4, TRAF6: TNF receptor associated factor 6, TAK1: TGF-β activated kinase 1, AP1: activating protein-1, RIP1: receptor-interacting protein 1, RIP3: receptor-interacting protein 3. (Adapted from ref 5)

Receptor-Ligand interaction

Although TLRs share a common architecture, they differ in the structural arrangement between their receptors. Upon their interaction with the ligands, TLRs will either dimerize into homo or heterodimers. TLR2 in association with TLR1 or TLR6 will form a hetrodimeric complex which will generate an internal hydrophobic pocket that is essential for lipoprotein binding. The TLR1/TLR2 complex recognises triacyl lipoproteins, whereas TLR2/TLR6 complex recognises diacyl lipoproteins that lack the amide-linkage. On the other hand, TLR3 forms a homodimer when binding to dsRNA ligand. TLR4 needs an accessory protein, also known as co-receptor, in order for it to bind to LPS. MD-2 is the co-receptor that is specific for gram-negative LPS recognition by the TLR4. First, MD-2 has to interact with TLR4 and alter its conformation, so TLR4-MD2 complex is now ready to sense its LPS ligand. The TLR4-MD2 complex will interact with the LPS in two regions. TLR4 binds to the phosphate group of the LPS; at the same time MD-2 binds to the lipid chain of the LPS resulting in a symmetrical homodimer complex.

Regardless of the minor differences on how TLRs interact with their ligands, the ligand-receptor interaction will eventually form an m-shaped dimeric structure that is important just to bring the intracellular TIR domains close to each other, preparing them for the signaling process.

Signaling pathways of TLRs

Signal transduction pathways activated by TLRs contribute to the induction of various pro-inflammatory cytokines and chemokines that are required for initiating the immune response. When TLRs bind to their ligands and dimerisation occurs, TIR domain will activate the downstream signaling by recruiting adaptor proteins. The first identified adaptor protein that

is used in almost all signaling pathways of TLRs (with the exception of TLR3) is the myeloid differentiation factor 88 (MyD88). Other known adaptor proteins are TIR-related adaptor protein inducing interferon (TRIF), MyD88-adaptor like (Mal), TRIF-related adaptor molecule (TRAM), and the sterile a- and HEAT/Armadillo motif containing protein (SARM). Depending on the type of adaptor used, TLR signaling is mainly divided into the MyD88-dependent pathway, or the TRIF-dependent (MyD88-independant) pathway.

MyD88 consists of two parts: TIR domain and the death domain (DD). In MyD88-dependent pathway, signaling starts when the TIR domain of the adapter protein binds to the TIR domain of the TLR forming TIR-TIR bridge. Then IL-1R associated kinase 4 (IRAK-4) interacts with MyD88 via its DD. Following that, IRAK-4 phosphorylates IRAK1 and IRAK2, which in turn activates TNF receptor associated factor 6 (TRAF6).

TRAF6 is an E3 ubiqutin ligase that can activate TGF-β activated kinase 1 (TAK1) in association with two other proteins TAK1 binding protein 1 (TAB1) and TAB2. TRAF6 itself forms a polyubiquitin that serves as a platform joining the two proteins TAB1 and TAB2. After that, TAK1 activates a series of MAP kinases 3 and 6, at the same time phosphorylates IKKβ (NF-κB essential modulator) (Figure 3). Beginning with MAPK3 and MAPK6, their activation results in phosphorylation of Jun kinases (JNKs) and p38; phosphorylated JNKs stimulate the transcription factor AP-1 (activating protein-1). Back to IKKβ; its phosphorylation by TAK1 contributes further to the phosphorylation of IκB, leading to the activation of another transcription factor NF-κB. The resultant transcriptional factors from the MyD88-dependent pathway are AP-1 and NF-κB that contribute to gene transcription of pro-inflammatory cytokines including TNFα and IL-12. Unlike other TLRs, TLR3 and TLR4 with the help of the adaptor protein TRAM signal through the TRIF-dependent pathway. TRIF is connected to three parts, TRAF3, TRAF6, and receptor-interacting proteins 1 and 3 (RIP1 and RIP3). TRAF6 associates with RIP1; together they activate NF-κB and MAPKs to induce gene transcription of cytokines, but not exclusively through the TRIF-dependent pathway. TRAF3, which is linked to both TBK1 and IKKε, phosphorylates the transcriptional factor IRF3 leading to the production of INF_{β}.

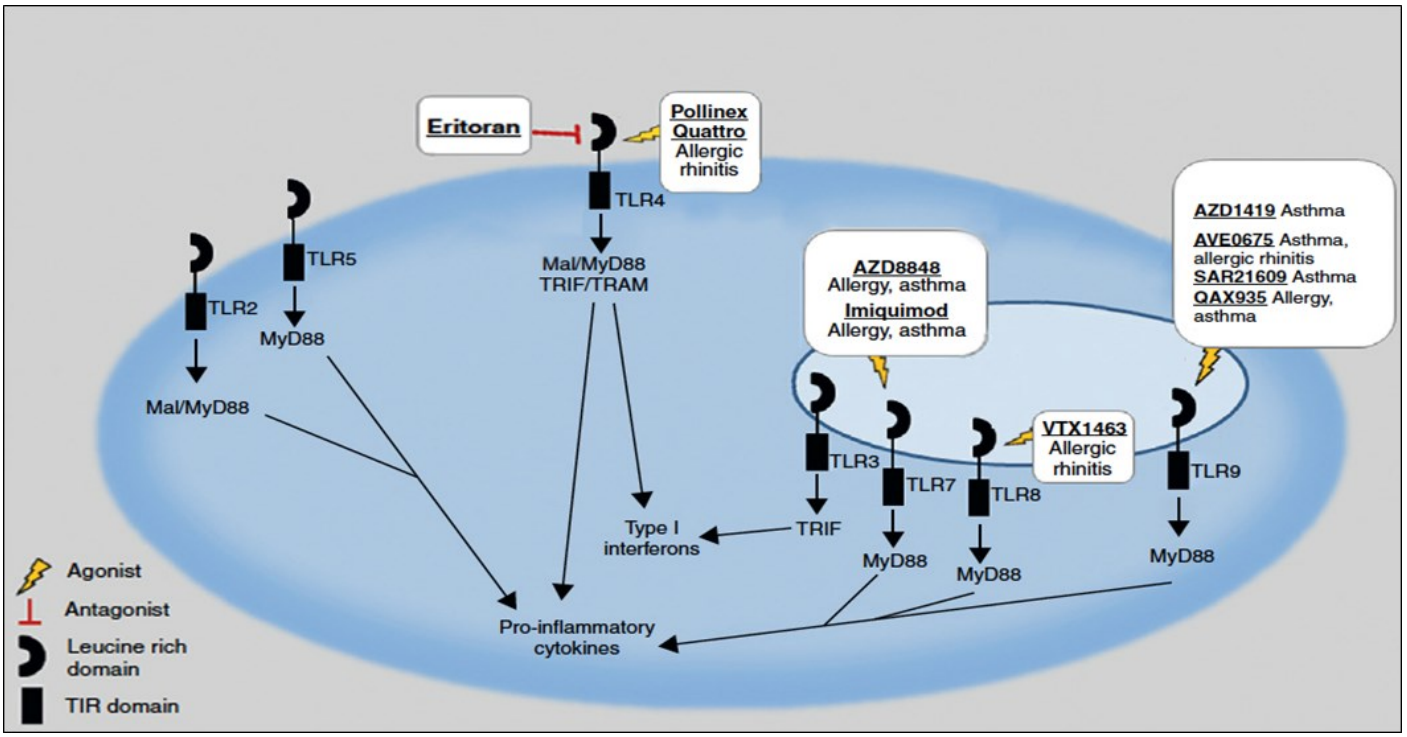

Figure 4. TLR-targeted therapeutics for asthma and rhinitis (adapted from ref 7)

Role of TLRs in the immune system

Toll-like receptors play a crucial role in recognising microbes of different origins. They are responsible for bridging the innate and the adaptive immune systems. Initially, when the host faces a pathogenic microorganism, the innate-immune system, through the pattern-recognition receptors (ex. TLRs), will imme-

diately recognize the pathogens. However, after few days when the initial response fails to get rid of the invading microorganism, the immune system will import an additional specific response that deals with the pathogen to ensure proper elimination of it, the so called adaptive/acquired-immunity. This is achieved by maturation of DCs that secrete proinflammatory cytokines such as TNF and IL-12, which in turn facilitate clonal expansion of T and B cells (cell components of the acquired-immunity). Thus, TLRs bring specificity to the innate-immune system. In studies linking TLR genetic variations to diseases, the first genetic variation was identified as a single nucleotide polymorphism (SNP) in TLR4, where two of the amino acids in the LRR were changed. This change results in impaired interaction of the receptor with the LPS, hence individuals become highly susceptible to sepsis when infected with gram-negative bacteria.

Role TLRs in allergic diseases

In respiratory airways, microorganisms and allergens are encountered by a wide variety of cells that are known to express TLRs; these cells include epithelial cells, DCs, macrophages, mast cells and eosinophils. TLRs play a pivotal role in evoking the appropriate response needed for protection against infectious and non-infectious diseases. It is now well established that genetic polymorphism involving TLR2 and CD14 (a co-receptor protein needed for TLR4 signaling) predisposes individuals to develop allergic asthma and atopy.

There is a strong correlation between asthma and TLR6; genetic variation in TLR6 contributes to the pathogenesis of asthma. Similarly, a study done on patients suffering from AR showed that TLR9 expression is greatly reduced in mucosal lining when compared to controls, which indicates that these patients have a decrease in their nasal innate defense (TLRs).

Despite their critical role in the respiratory system, TLRs can be a double-edged weapon depending on which receptor will be activated. For example, activation of certain types of TLRs can promote sensitisation and breakdown of tolerance, whereas activation of others can possibly encourage tolerance even to inoffensive aero-allergens especially if it occurs early in life. In case of asthma, activation of certain TLRs like TLR3 or TLR4 upon contact with infectious microbes can exacerbate airway hyper-responsiveness (AHR) and promote asthma attack. On the other hand, activation of TLR7 and TLR9 has protective effects in asthma and AR. Genetic studies on patients suffering from AR found that SNPs in certain TLRs can render them functionally active or non-active, thus the receptor can become either protective or detrimental. For example, polymorphisms in TLR2, TLR4 and TLR10 protect against AR, whereas polymorphisms in TLR7 and TLR8 increase the chance to develop AR.

TLRs as therapeutic targets in allergic diseases (asthma and rhinitis)

In general, TLRs fulfill the criteria for being good therapeutic targets for many conditions including allergic diseases (6). TLRs are the first component to be activated during the inflammatory cascade; hence at this earlier point, it would be beneficial if we target them to modulate the immune response and control the inflammation (7). TLRs are over-expressed in diseases and can be targeted clinically using antibodies, small molecules and nucleic acid-based drugs, some of which are currently in clinical trials (Table 2 and Figure 4).

Clinical agents targeting TLR4, TLR7, TLR8 and TLR9 are listed below.

TLR-4

In asthma and AR, TLR4 can be targeted with agonists or antagonists. TLR4 is activated with agonists as an adjuvant in allergy vaccine, whereas it is inhibited with antagonists to reduce its expression and inflammatory response.

Agonists

Pollinex Quattro, developed by Allergy Therapeutics, is a well-tested allergy vaccine that is designed for an optimal control of seasonal allergic rhinitis symptoms (8). It is currently in phase III clinical trials (6). The vaccine consists of three components, a modified L-tyrosine chain, monophosphoryl lipid A (MLA) and allergens (6,8). The modified L-tyrosine chain is adsorbed into the allergen that could be grass, flower, ragweed or a tree pollen extract. Then the two components bind to the MLA, a monophosphoryl lipid A which is a detoxified product obtained from LPSs of *Salmonella minnesota* cell wall (8). This LPS byproduct is a strong TLR4 agonist that activates and triggers the signaling pathways through the receptor (7).

Pollinex Quattro hope that the allergen along with the ligand are introduced to be recognised by the TLR4; by this technique the immune system is directed to become familiar and tolerant to the allergen, hence reducing the severity of the seasonal allergies (6-8). In a study evaluating the clinical efficacy of Pollinex, it was shown that patients treated with the vaccine prior to the seasonal allergy experienced a great improvement in the disease course and the effects lasted for up to 5 y (8). The clinical improvement is attributed to the decrease in IgE levels and induction of T-regs. Beside the clinical improvement that is experienced by the patients, their immune profile after the immunotherapy with the vaccine was dramatically changed due to the induction of Th1 response (9). It is of interest to note that Pollinex is a safe and well tolerated vaccine without significant reported side effects apart from the local reaction of subcutaneous injection (8).

Antagonists

In mouse models of established airway allergy, administration of TLR4 ligand (LPS) was shown to exacerbate AHR, inflammation and remodeling (10). Eritoran, a TLR4 antagonist, was first tested in murine models for septic shock; later it was also studied in mouse models of airway diseases, asthma and COPD (11). Mice treated three times weekly with eritoran in a dose of 50 µg for 5 weeks and then exposed to daily inhalation of LPS, revealed reduction in neutrophil levels, and AHR when challenged with metacholline. Thus, eritoran shows beneficial effects in the treatment of asthma (11).

TLR-7 and TLR-8

Synthetic agonists targeting TLR7 and TLR8 show beneficial effects in the treatment of asthma and AR (12). In ovalbumin-induced allergic asthma, administration of TLR7 agonist showed a reduction in airway resistance and hyper-responsiveness in the murine models. AZD8848, a compound developed by AstraZeneca, is a small molecule that acts as an agonist for TLR7 (13), and is currently undergoing phase II clinical trials (7). This compound exerts its effects by reducing mast cell activity and producing IL-10, which eventually suppresses the allergic symptoms associated with bronchial asthma and AR. The side effects of AZD8848 are minimal and are mostly dose dependent. Nasal symptoms include rhinorrhea, blockage, epistaxis and influenza-like symptoms (14). Another agonistic compound that targets TLR7 is imiquimod (R837), which has been approved for the treatment of genital warts, actinic keratosis and basal cell carcinoma. Imiquimod has shown promising effects in the treatment of asthma.

VTX-1463 is a ssRNA-based molecule currently in phase II clinical trials (12), produced by VentiRx pharmaceuticals and is classified as an TLR8 agonist. It was shown that patients with AR when sensitized with grass pollen and then treated with a course of five doses of intranasal VTX-1463 showed dramatic reduction in their nasal symptoms without any reported side effects (8). The improvement in nasal symptoms is believed to be due to the ability of VTX-1463 to shift from Th2 response (primary response in allergic diseases) into Th1 response (7). Mediating Th1 response stimulates the production of IL-12 and IFNγ cytokines that suppress the allergic reaction.

Another potential therapeutic agent is resiquimod, (R848), a dual agonist targeting both TLR7 and TLR8, sharing a common structural homology with imiquimod. This compound is in phase I clinical trials for the treatment of asthma and AR (7, 13).

TLR-9

TLR9-targeted therapeutics in the clinical development for allergies has shown promising results (7, 12). AVE0675, SAR21609 and QAX935 are synthetic CpG oligonucleotides that resemble unmethylated CpG of bacterial DNA. These agents function as TLR9 agonists that induce a strong Th1 response manifested as increase in IL-10 and IFN-γ levels. In addition, decrease in the levels of IgE, IL -4, IL-5 and eosinophils were also reported. Furthermore, intra-nasal inhalation of QAX935 in monkeys led to a decrease in airway inflammation, resistance and remodeling. The three agents have demonstrated long-lasting effects that could be considered for treatment and prophylaxis of asthma and AR (15). TLR9 agonists are in phase I clinical trials and further investigations in their efficacy and safety are needed.

Similar to Pollinex Quattro, AIC, produced by Dynavax, is another example of allergy vaccine for AR (16). It is currently in phase II clinical trials (8). AIC is an allergen-1018ISS conjugate, where 1018ISS is a potent TLR9 agonist. Following administration of AIC to a group of mice sensitised to ragweed, an increase in Th1 and Treg cytokines was reported (17). Furthermore, switching from

Table 2. Summary of TLR-targeted therapeutics in clinical development of allergic diseases.

Drug	Target	Company	Allergy	*
Pollinex Quattro	TLR4 agonist	Allergy Therapeutics	Allergic rhinitis	3
AZD8848	TLR7 agonist	Astra Zeneca	Asthma/ rhinitis	2
VTX-1463	TLR8 agonist	VentiRx Pharma	Allergic rhinitis	2
R848	TLR7/8 agonist	3M Pharmaceutics	Asthma	1
AVE0675	TLR9 agonist	SanofiAventis /Coley Pharma	Asthma/ rhinitis	1
SAR21609	TLR9 agonist	Sanofi Aventis/Coley Pharma	Asthma/ rhinitis	1
QAX935	TLR9 agonist	Idera Pharma	Asthma/ rhinitis	1
AIC	TLR9 agonist	Dynavax	Allergic rhinitis	2

*Phase of clinical trials

IgE antibody production into IgG type was also detected (18). In one clinical study investigating the efficacy of AIC, allergic participants were treated with the vaccine prior to the ragweed season in 2001 and followed until the next ragweed season of 2002 (18,19). During the period of follow up, nasal and chest symptoms (tightness, wheezing, and cough) were alleviated (18). Local and systemic side effects of AIC were minimal and none of them required medication (19).

Conclusion

In conclusion, TLRs represent a major class of pattern-recognition receptors (PRRs) that recognise various types of microorganisms, leading to production of inflammatory cytokines. Their role in the immune system, including the lung, is essential for the host defense, yet when they fail to discriminate between self and non-self-molecules, a disease condition results. The link between TLRs and allergic disease suppression is well established by several clinical studies. Targets for TLRs 4, 7, 8 and 9 have shown positive results in the treatment of asthma and AR. Although further data is required to confirm their usefulness, the field of TLRs is very promising and may yield novel drugs in the near future.

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TEST YOUR KNOWLEDGE

Answers on back page

1) *Which of the following is a cell surface TLR?*
   a) TLR 4
   b) TLR 5
   c) TLR 1
   d) TLR 2
   e) All of the above

2) *Which TLR, located in the endosome, can sense foreign nucleic acids within the cell?*
   a) TLR 2
   b) TLR 6
   c) TLR 8
   d) TLR 5
   e) TLR 4

3) *Which of the following is the ligand of TLR 5?*
   a) dsRNA
   b) Lipo polysaccharides
   c) Bacterial flagellin
   d) Profilin-like protein
   e) Diacyl lipoproteins

Is there a problem?

A 25 year old female patient was given the following prescription for mild urinary tract infection. She is one week pregnant and not allergic to any antibiotics. Is there any major error with the prescription?

Patient Name: Fatima Ahmad Age: 25 years
Address: Street No.12

Rx

Doxycycline capsules
200 mg once daily x 3 days
Send one pack

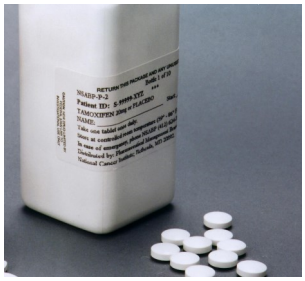
Dr. Ahmad
Signature Date: 1/03/16

Answer (Prescription Exercise)

Doxycycline is contraindicated in pregnancy.

Source: British National Formulary

TOPICAL ISSUES AND CONTROVERSIES



Preventive tamoxifen lowers rate of ER+ breast cancer

Tamoxifen given to women at high risk for breast cancer significantly lowered the rate of breast cancer diagnosis in the International Breast Cancer Intervention Study-I (IBIS-I) trial. The reduction in breast cancer incidence held up over a median 16-year follow-up period.

Tamoxifen resulted in a 29% reduction in breast cancer compared with placebo, and the rates of estrogen receptor (ER)-positive breast cancer were reduced by 35%. Tamoxifen had no effect on ER-negative breast cancers. Tamoxifen is the only clear case in which preventative activity will produce such a long benefit as compared to aspirin in cancer prevention.

There were concerns about increased endometrial cancer risk, and patients also had a higher incidence of non-melanoma, low mortality–risk skin cancers. Nine cases of endometrial cancers occurred during the active treatment window but the endometrial cancer risk was statistically non-significant, meaning that it could be a chance observation. Rates of colorectal cancers were lower in the tamoxifen-treated group compared with the placebo group. Women in the tamoxifen group had a small, non-significant increase in all-cause mortality.

The IBIS-I trial randomized 7,154 pre- and post-menopausal women to receive either a daily 20 mg dose of tamoxifen for 5 years or placebo. Women in the study had a high risk of breast cancer, due mostly to a family history of the disease, and were all between the ages of 35 and 70. On average, women were 50.8 years of age when they entered the study, and about 54% were post-menopausal in both trial arms.

After 10 years, 4.6% of women in the tamoxifen arm were diagnosed with breast cancer compared with 6.3% in the control arm—a 28% reduction in incidence. Fifty-nine women had to be treated to prevent a single case of breast cancer.
After 20 years, 3.3% of women in the tamoxifen arm were diagnosed with breast cancer compared to 6.3% in the control arm—a 30% reduction in overall incidence. Twenty-two women had to be treated to prevent a single case of breast cancer over the 20-year period. "This is very favorable compared to any preventive treatment for heart disease or other diseases.
Whether patients were on hormone replacement therapy (HRT) turned out to be important, as only those women not on HRT had a beneficial effect from tamoxifen. Women who did not take HRT had a 45% reduction in ER-positive disease and 38% reduction in breast cancer overall.
Prior tamoxifen showed clear benefits of about 30-35% in the first 10 years of follow-up. The current trial is unique in its long-term follow-up of patients-most patients were followed for almost 20 years with the longest being 22 years. With a current median age of 66, there is still substantial follow-up remaining for the women on this trial.

Reference

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Marijuana compound a novel treatment for Alzheimer's ?

Extremely low levels of delta-9 tetrahydrocannabinol (THC), the active compound in marijuana, may offer a novel and viable treatment for Alzheimer's disease (AD).

Investigators at the University of South Florida in Tampa found that THC both decreases the production of amyloid beta (Aβ) and inhibits its aggregation in cell cultures and enhances mitochondrial function as well. And it does so at extremely safe doses suggesting a potentially new therapeutic approach for AD).

Amyloid lowering

Investigators incubated THC together with a variant of Aβ protein precursor cells and assayed the culture for the presence of Aβ levels at 6, 24, 48h. TCH was also tested for synergy with caffeine to see whether the combination led to greater reductions in the Aβ levels of these Aβ protein precursor cells *in vitro.*

THC was shown to lower Aβ levels in [Aβ protein precursor cells] at extremely low concentrations. However, the combination of TCH and caffeine was not synergistic.

The same active compound in marijuana directly interacted with Aβ peptide, thereby inhibiting its aggregation. Low doses of THC enhanced mitochondria function as well.

Previous research by this team has shown that Aβ migrates into the mitochondria with age, impairing its function.

Neuroprotective effect

Concerns about memory impairment are frequently raised in the context of any research associated with potential therapeutic benefits of THC.

However, memory impairment is only observed at "abuse" concentrations of THC, which are more than a thousand times higher than the doses used in this study.

Newer research also suggests that such ultra-low doses of TCH have a neuroprotective effect in the earlier stages of Alzheimer's.

Both early-onset familial AD as well as late-onset sporadic AD are characterized by extracellular Aβ peptide and by amyloid plaques along with tau-containing neurofibrillary tangles.

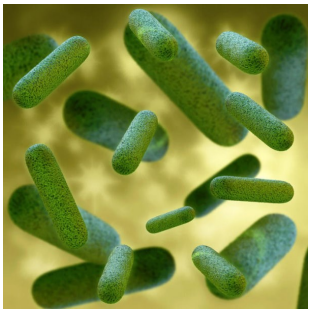
The continuous aggregation of Aβ peptides along with hyper-phosphorylation of the tau protein inside the cell causing neurofibrillary tangle formation are generally accepted as the major etiologic factors behind the neuronal cell death associated with AD progression.

The interrelationship between neurodegeneration

and the role of cannabinoids is intricate and complex. Given that this THC 'dose' is much less than what is used recreationally, there is a possible place for cannabinoid therapy in Alzheimer's.

Reference:
J Alzheimers Dis. Published online August 27, 2014.

Bacteria use plan to evade antibiotics



Bacteria rely on an enzyme called type I signal peptidase (SPase) to secrete a diverse range of proteins, including those required for evading host immune response and scavenging nutrients from the environment. Because of its key role in bacterial survival, SPase is a promising drug target.

Natural products called arylomycins are potent inhibitors of SPase that are currently being developed as antibiotics. But resistance rates remain high in Gram-positive bacteria, including *Staphylococcus aureus*. In a recent[1] study *S. aureus* was shown to resist the effects of arylomycins (1). Apparently, bacteria shift to a back-up plan when protein secretion is prevented, allowing them to bypass the need for SPase. According to the authors, the findings have important implications for protein secretion in bacteria and potentially for protein trafficking in general.

SPase's job is to clip peptide sequences off proteins as they pass from the inside of a cell to the outside. In another report, the same authors found that that *S.aureus* responds to arylomycin-mediated SPase inhibition by increasing expression of four adjacent genes, *SA0337* to *SA0340*[2]. In addition, arylomycin resistance was conferred by loss-of-function mutations in *SA0337*.

These four genes constitute an operon- a functioning unit of genomic DNA containing a cluster of genes under the control of a single promoter. *SA0337* serves as a transcriptional repressor to control the operon's expression. Under normal conditions, the operon is repressed by *SA0337*, but it is released from repression by SPase inhibition.

The protein products of the downstream genes together then confer resistance to arylomycin M131 by providing an alternate and novel method of releasing translocated proteins, thereby bypassing the essentiality of SPase. Each member of the operon was required in order to tolerate SPase inhibition. The authors named the repressor gene*ayrR* and the downstream genes *ayrABC* for their role in arylomycin resistance. They also demonstrated that AyrABC is able to mediate secretion of the proteins normally processed by SPase, although in some cases with reduced efficiency.

It is still not clear why bacteria evolved this alternative pathway. According to the authors, the natural physiological role of the pathway may be to support SPase function when elevated secretion is required and the capacity of SPase has been exceeded. On the other hand, it may have evolved to facilitate survival in the presence of an SPase inhibitor. After all, SPase is exposed on the outer surface of the cytoplasmic membrane, making it uniquely susceptible to such inhibitors. Moreover, the evolution of four different families of arylomycins further suggests that SPase inhibition may have represented a significant selection pressure, as does the existence of multiple, redundant SPases in many Gram-positive bacteria.

This study indicates that SPase is not a good target for the development of new antibiotics because microorganisms can readily escape by using an alternative means of release. So far, Romesberg's group have only shown that the process exists and have identified at least some of the genes involved. The next step is to find how the bacteria sense that there is a problem with secretion and how they then relay this information to genes that control the alternate process. This not only will elucidate important aspects about how bacteria survive and respond to different types of stress, but it might eventually help design antibiotics that do not induce the process.

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NEWS from the FDA

Blinatumomab for acute lymphoblastic leukemia

In December 2014 FDA approved Blinatumomab (Blincyto) for Acute Lymphoblastic Leukemia; the first approved anti-CD19 agent. Approval was given for treating patients with Philadelphia chromosome (Ph)-negative precursor B-cell acute lymphoblastic leukemia (ALL), a rare form of the disease wherein a patient's bone marrow makes too many B-cell lymphoblasts. The drug is intended for patients with relapsed or refractory disease. Blinatumomab is a "bispecific CD19-directed CD3 T-cell engager that activates endogenous T cells when bound to the CD19-expressing target cell,"

The FDA worked proactively with the sponsor under their breakthrough therapy designation programme to facilitate the approval of this novel agent. The NCI estimated about 6,000 patients would be diagnosed with ALL in 2015, with 1,440 expected mortalities. The trial that led to the approval of blinatumomab (Protocol MT103-211) was a multicenter, single-arm trial that involved 185 adults with relapsed or refractory Ph-negative precursor B-cell ALL. Patients received blinatumomab, administered by continuous infusion for 4 weeks of a 6-week cycle. Up to 2 cycles were used for induction and 3 cycles for consolidation. About 32% of participants achieved complete remission for 6.7 months (0.46–16.5 months). Thirty-one percent of patients achieved complete remission with or without complete hematologic recovery but with reduction in minimal residual disease to less than 10-4.The most common adverse events were constipation (20%), tremor (20%), rash (21%), hypokalemia (23%), peripheral edema (25%), febrile neutropenia (25%), nausea (25%), headache (36%), and pyrexia (62%). Neurologic toxicity occurred in approximately 50% of patients and was a frequent cause of therapy interruption. Cytokine release syndrome was reported in 11% of the patients—these included life-threatening or fatal events. Blinatumomab carries a boxed warning regarding this syndrome and neurologic toxicities. As the drug comes with serious risks and the potential for errors in administration and preparation, the FDA has approved blinatumomab with a Risk Evaluation and Mitigation Strategy.

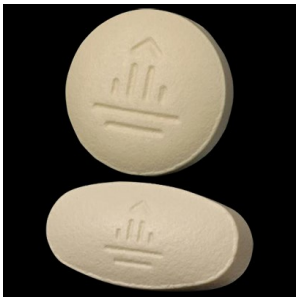
Empagliflozin for Type 2 diabetes

Empagliflozin produced sustained glycemic and weight control in type 2 diabetics during a 78-week open-label extension study. And the drug was well tolerated, with a low risk of hypoglycemia. Empagliflozin inhibits sodium glucose co-transporter 2 (SGLT2), which is located in the proximal tubule of the kidney. SGLT2 inhibitors lower blood glucose by reducing reabsorption of glucose filtered by the kidneys and by increasing urinary glucose excretion.

The current study is an extension of two 12-week, blinded dose-finding studies comparing 10 or 25 mg of empagliflozin, as monotherapy or as an add-on to metformin, to open-label comparators.

At week 90, the reductions from baseline HbA1c were 0.34% to 0.63% with empagliflozin; 0.56% with metformin; and 0.40% with sitagliptin.

Mean weight loss from baseline was 2.2 to 4 kg with empagliflozin, 1.3 kg with metformin, and 0.4 kg with sitagliptin.



Most adverse events were mild or moderate. Genital infections occurred in 3- 5.5% of the patients on empagliflozin, 1.8% of patients on metformin, and none of the patients on sitagliptin. Urinary tract infections occurred in 3.8-12.7% of patients on empagliflozin, 3.6% of patients on metformin, and 12.5% of patients on sitagliptin.

In conclusion, empagliflozin is safe and efficacious, perhaps not as effective compared to metformin as this study indicates, in the short term but further studies need to be conducted to prove its efficacy and safety on the long term.

Source

www.medscape.com/viewarticle/815349

FDA approves Pembrolizumab for advanced melanoma

The FDA granted accelerated approval to the anti-programmed cell death protein 1 (PD-1) drug pembrolizumab (Keytruda) for the treatment of melanoma patients with relapsed or refractory disease. Pembrolizumab (formerly MK-3475) becomes the first approved anti-PD-1 drug and is intended for use following treatment with ipilimumab, or after treatment with ipilimumab and a BRAF inhibitor in patients who carry a BRAF mutation. Keytruda is the sixth new melanoma treatment approved since 2011, a result of promising advances in melanoma research. The FDA previously approved ipilimumab, vemurafenib, peginterferon alfa-2b, trametinib, and dabrafenib for the treatment of melanoma. The trial that led to the approval of pembrolizumab included 173 melanoma patients whose disease had progressed following treatment. All participants received the study drug, with patients randomized to receive the recommended dose of 2 mg/kg, or a higher dose of 10 mg/kg. The overall response rate in patients who received pembrolizumab at the 2 mg/kg dose was approximately 24%. The duration of response was at least 1.4 to 8.5 months, with most patients having a response that continued beyond that. The response rate at the 10 mg/kg dose was comparable to the lower dose. The most common adverse events reported in patients who received pembrolizumab were pruritus, rash, fatigue, nausea, cough, constipation, arthralgia, decreased appetite, and diarrhea. Though uncommon, the drug also carries the potential for severe immune-mediated adverse events involving the lungs, liver, colon, and hormone-producing glands.

Source
http://www.cancernetwork.com/news

FDA approves triple-combination HIV pill Triumeq

The FDA approved the once-daily fixed-dosetriple-combination pill *Triumeq* (ViiV Healthcare) for the treatment of HIV infection in adults and adolescents aged 18 years and older, according to the manufacturer.

Triumeq combines an integrase strand transfer inhibitor (dolutegravir, 50 mg) with 2 nucleoside reverse transcriptase inhibitors (abacavir, 600 mg, and lamivudine, 300 mg).

Before initiating treatment with abacavir-containing products, screening for the presence of a genetic marker, the HLA-B*5701 allele, should be performed in any HIV-infected patient, irrespective of racial origin. Abacavir should not be used in patients known to carry the HLA-B*5701 allele. Patients who carry this genetic marker are at high risk of experiencing a hypersensitivity reaction. The label contains a boxed warning regarding the risk for hypersensitivity reactions to abacavir products, lactic acidosis and severe hepatomegaly, and exacerbations of hepatitis B.

The EC and FDA cleared Triumeq based primarily on results from 2 clinical trials, including a phase 3 study of treatment-naive adults, in which 80% of patients achieved virological suppression of the virus with dolutegravir and abacavir/lamivudine (given separately) vs 72% of those taking efavirenz, emtricitabine, and tenofovir (*Atripla*, Bristol-Myers Squibb and Gilead Sciences), according to the ViiV news release.

Grade 2 to 4 treatment-emergent adverse reactions occurring in 2% or more participants taking the dolutegravir-based regimen were insomnia (3%), headache (2%), and fatigue (2%), the company says.
Once-daily dolutegravir was approved in the USA as monotherapy (under the name Tivicay) in August 2014 and in Europe in January 2014.

Triumeq alone is not recommended in patients with resistance-associated integrase substitutions or clinically suspected integrase strand transfer inhibitor resistance because the dolutegravir dose in the combination is insufficient for those individuals,

Source
http://www.medscape.com/viewarticle/831125?nlid=65023_1842&src=wnl_edit_medp_wir&spon=17

Drug granted breakthrough therapy designation for Refractory Renal Cell Carcinoma

Patients with renal cell carcinoma (RCC) who have not responded to prior therapy may soon have a new treatment option. It is a drug already approved for progressive, metastatic medullary thyroid cancer (MTC). On August 24, 2015, the FDA granted Breakthrough Therapy Designation for cabozantinib as a potential treatment for patients with advanced RCC who have received one prior therapy.[1] Cabozantinib inhibits the activity of tyrosine kinases including MET, VEGF receptors, AXL, and RET and may help improve overall survival in patients who have experienced disease progression following treatment with a VEGF receptor tyrosine kinase inhibitor (TKI). Drugs that receive Breakthrough Therapy Designation may benefit from involvement of FDA senior managers in the review process, potential rolling submission and/or Priority Review of a sponsor's New Drug Application (NDA), and other benefits.

The Breakthrough Therapy Designation was based on the results of METEOR, a pivotal phase III trial comparing cabozantinib to everolimus (Afinitor) in patients with RCC who experienced disease progression following treatment with a TKI. METEOR met its primary endpoint and demonstrated a statistically significant increase in progression-free survival (PFS) for cabozantinib as compared to everolimus in the first 375 patients randomized. The study demonstrated that cabozantinib reduced the rate of disease progression or death by 42% compared to everolimus.

This agent currently is marketed in capsule form under the brand name COMETRIQ® in the US for the treatment of progressive MTC, and in the European Union for the treatment of adult patients with progressive, unresectable locally advanced, or metastatic MTC.

The majority of clear cell RCC tumors exhibit down-regulation of von Hippel-Lindau (VHL) protein function. This results in a stabilization of the hypoxia-inducible transcription factors and consequent up-regulation of VEGF, MET, and AXL. The up-regulation of VEGF may contribute to the angiogenic nature of clear cell RCC. It is believed that the expression of MET or AXL may be associated with tumor cell viability, a more invasive tumor phenotype, and reduced overall survival.

If detected in its early stages, the 5-year survival rate for RCC is high. However, the 5-year survival rate for patients with advanced or late-stage metastatic RCC is under 10%, according to the American Cancer Society (ACS).[2] Clinicians have been disappointed in recent years because the currently approved agents have shown little differentiation in terms of efficacy and have demonstrated only modest PFS benefit in patients refractory to sunitinib, a commonly used first-line therapy.

The most commonly reported adverse drug reactions (≥25%) reported with this agent are diarrhea, stomatitis, palmar-plantar erythrodysesthesia syndrome (PPES), decreased weight, decreased appetite, nausea, fatigue, oral pain, hair color changes, dysgeusia, hypertension, abdominal pain, and constipation. The most common laboratory abnormalities (≥25%) are increased AST, increased ALT, lymphopenia, increased alkaline phosphatase, hypocalcemia, neutropenia, thrombocytopenia, hypophosphatemia, and hyperbilirubinemia.

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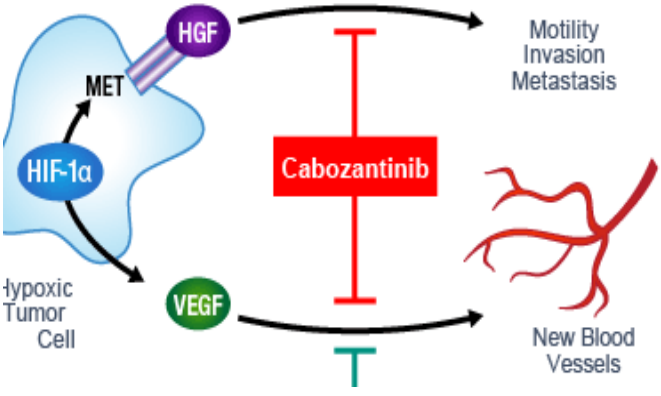


ISLAMIC CONTRIBUTIONS TO MEDICINE

With the decline of the Western Roman empire, Europe entered its medieval era, the so-called 'Dark Ages' between the 6th and 13th centuries, a period of economic and intellectual wilderness between the 'light of Rome' and the rise of the Italian renaissance. But elsewhere, in the heartlands of the Islamic empires, stretching from Spain and the Maghreb in the West to the borders of China in the East, it was the 'Age of Enlightenment', a time of great achievement in the arts and in scientific inquiry.

Just as Greco-Roman philosophy served as a common intellectual framework for professional medical practice in the Islamic Near East, so Arabic medical literature of the 8th to 12th centuries, through Latin translations, provided early renaissance Europe with ideas and practices from which early modern medicine eventually arose.

Greek medical teachings were preserved and valued in the diverse muslim lands for dealing with medical problems common to all peoples. This heritage of medical theory and practice, mingled with Persian, Indian and Arab elements, was assimilated and elaborated by a community of both muslim and non-muslim physicians speaking many languages; Arabic, Persian, Syriac, Hebrew and Turkish, though Arabic became the lingua franca and Islam the dominant faith.

As cosmopolitan Islamic culture developed, Islamic medicine has shown great variation and diversity, as shared traditions spanned vast areas and crossed many centuries. The institutions and policies responsible for dispensing medical care were subject to political and social fluctuations in different regions: the dietary and fasting laws and the general rules for hygiene and burial of the different religious communities of muslims, jews, christians, zoroastrians, and others; the climatic conditions of the desert, marsh, mountain and littoral communities; the living conditions of nomadic, rural, and urban populations; local economic factors and agricultural successes or failures; population migration as well as travel undertaken for commerce and pilgrimage; the injuries and diseases attendant in war; the incidence of plague and other epidemics as well as the occurrence of endemic conditions such as trachoma and other eye diseases.

Medical care is, in addition, always multifaceted, with the needs of the society being served by various local traditional practices as well as the formal learned medicine. The sophisticated learned Islamic medical texts represent only one facet of the actual medical care of the society. The economic and social level of the patient determined to a large extent the type of care sought, and the expectations of the patients varied as did the approaches of the medical practitioners.

Folio from an Arabic manuscript of Dioscorides De Materica Medica, 1229

The medical care in the medieval Islamic lands involved a rich mixture of religions and cultures to be seen in both the physicians and the patients, a co-existence and blending of traditions probably unrivaled in contemporaneous societies. The medical profession in general transcended the barriers of religion, language and country.

Greek influences

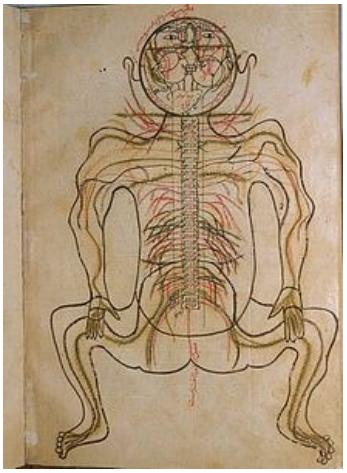
The medical theories inherited particularly from the Greek world supplied a thread of continuity to professional learned medical practice throughout the medieval Islamic lands. The caliphs al-Mansur, Harun al-Rashid and al-Ma'mun are noted for their patronage of learning and medicine. When suffer-

ing from a stomach complaint, al-Mansur, who ruled from AD 734 to 775 (158-169 AH), called a christian Syriac-speaking physician Jurjis ibn Jibra'il ibn Bakhtishu`to Baghdad from Gondeshapur in southwest Iran. His son was also called to Baghdad in AD 787 (171 AH), where he served as physician to Harun al-Rashid. For eight generations, well into the second half of the 11th century, twelve members of the Bakhtishu` family were to serve the caliphs as physicians and advisors, to sponsor the translation of texts, and to compose their own original treatises. A remarkable, if not unique record, in the history of medicine.

Early in the 9th century, there was established in Baghdad a foundation called the House of Wisdom (*Bayt al-Hikmah*), which had its own library. Its purpose was to promote the translation of scientific texts. The most famous of the translators was Hunayn ibn Ishaq al-`Ibadi, a Syriac-speaking christian originally from southern Iraq. He was the author of many medical tracts and a physician to the caliph al-Mutawakkil (AD 847-861/232-247AH), but he is most remembered as a translator. He produced a truly prodigious amount of work before his death in about AD 873 (260 AH), for he translated nearly all the Greek medical books known at that time, half of the Aristotelian writings as well as commentaries, and various mathematical treatises. Ten years before his death he stated that of the works of Galen (Claudius Galenus; a prominent Greek physician philosopher; cAD 129) alone, he had made 95 Syriac and 34 Arabic transcripts. He was no doubt responsible, more than any other person, for the establishment of the classical Arabic scientific and medical vocabulary. Through these translations a continuity of ideas was maintained between Roman and Byzantine practices and Islamic medicine.

A number of Hippocratic treatises circulated in Arabic translations made at this time, as well as the writings of more than a dozen other Greek physicians and some Syriac, Persian and Indian medical writers. Knowledge of medicinal substances was based initially upon the illustrated treatise on *materia medica* written in Greek by Dioscorides in the 1st century AD. Several Arabic translations and revisions of his treatise were undertaken in 9th-century Baghdad and 10th-century Spain and later.

No single figure was of greater influence upon medieval Islamic medicine than Galen. In his writings he displayed a firm belief in a spiritual providence and in the foresight and design of the Creator as exemplified in the human form; ideas acceptable to muslim physicians. The combination of philosophy and medicine, which is so evident in the writings of Galen, persisted as a part of medieval Islamic medical literature.



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Answers to: Test your knowledge

Correct answers:
1-e; 2-c; 3-c

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