Naive Cell

A mature cell (especially a lymphocyte) that is capable of performing its full range of functions but which has yet to be stimulated to respond to a specific antigen.

Lymphocytes

Naive T Cell

Mature T cell that has not been activated before.

Antigen Presentation via MHC Class II Molecules

Nasal-Associated Lymphoid Tissue

Nasal-associated lymphoid tissue (NALT) refers to secondary lymphoid tissue in the respiratory tract.

Mucosa-Associated Lymphoid Tissue

Natural Antibodies

Autoantigens

Natural Antibody

An antibody produced without apparent antigenic stimulation.

Autoantigens

Natural Killer Cell Assay

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Synonyms
Natural killer $^{51}$Cr release assay, non-radioactive flow cytometric analysis of NK cell cytotoxicity.

Short Description
The described tests are used as in vitro or ex vivo assays to measure cytotoxic activity of effector cells—here, natural killer (NK) cells—from peripheral blood or spleen. In immunotoxicologic investigations these assays are intended for detection of possible alterations in NK cell function as an effect of exposure to drugs and chemicals.

Characteristics

Principle
The principle of the tests is based on co-cultivation of effector cells (E) and target cells (T) followed by determination of the proportion of dead target cells. The E:T ratios used in the assays normally vary between 25:1 and 200:1.

Target Cells and Mechanism of Killing
NK cells play a central role in the innate immune defence against intracellularly infected cells and tumours, without possessing any memory cell function based on prior antigen exposure. NK cells kill their targets either by the well known secretory/necrotic cytotoxic mechanism, associated with perforin-mediated killing when eliminating rare leukemic cell lines ex vivo, or by non-secretory/apoptotic mechanisms, associated with killing of solid tumour cells ex vivo. The described NK assays measure NK cell killing of the first type using a lymphoid leukemic cell line, YAC-1, as a target for rat or mice NK cells, or a myeloid leukemic cell line, K562, as a target for human NK cells. To obtain a high sensitivity of the assays target cells...
need to be young and in the log phase of growth for efficient lysing.

**Effector Cells**

There are primarily two sources of effector cells: peripheral blood and spleen. The effector cells are prepared either as a mononuclear cell suspension by Ficoll separation of heparinized peripheral blood or by single-cell preparation of the spleen. For spleen cells, mechanical disaggregation is preferred since enzymatic treatment procedures might interfere with cell function. Effector cells should be prepared within 24 hours (preferably immediately) after sampling to minimize possible risks of secondary inhibitory effects on the cytolytic activity. It is recommended that effector cells are kept at room temperature, not being refrigerated or cryopreserved, because such treatment will cause loss in activity. All lymphoid cells in the peripheral blood or spleen preparations are referred to as “effectors” when establishing the different E : T ratios used in the assays. However, only approximately 5–15% of the lymphoid cells, in rats or humans, are NK cells by definition.

**NK Cell Activity vs Cell Number**

Determination of the NK cell proportion in the effector cell population can be performed by flow cytometric immunophenotyping using e.g. CD161 rat-specific antibodies, or CD16/CD56 human-specific antibodies. However, the NK phenotype is highly heterogenous and approximately 50 different subsets have been identified. All NK cells may not exhibit lytic activity against the target cell lines, thus a correlation between the total number of NK cells and measured cytotoxic activity is not always obvious. It should be noted that significant decreases in NK cell activity can be observed without any significant reduction in NK cell number (1). Therefore, phenotypic enumeration of NK cells and measurement of cytotoxic NK cell activity should be regarded as two separate parameters. Each laboratory should establish their own baseline values regarding normal cytotoxic activity, as this may vary between species, strains and compartment for isolation of effector cells.

**51Cr Release Assay**

The conventional $^{51}$Cr release assay (2) is based on radiolabeling of target cells with $^{51}$Cr. After 4 hours co-incubation with effector cells the amount of $^{51}$Cr released into the supernatant is quantified in a gamma counter. The endpoint shows an indirect proportion of killed target cells given as percentage specific cytolyis and is calculated by the formula:

$$\left(\frac{\text{Release in experimental sample} - \text{spontaneous release}}{\text{Total release} - \text{spontaneous release}}\right) \times 100$$

The method is limited by spontaneous leakage of $^{51}$Cr from the target cells that increases with time. After 24 h the spontaneous release is $> 50\%$. Approximately $1 \times 10^6$ effector cells are needed per sample using an E:T ratio of 100:1. Therefore, only splenic effectors will be numerous enough for rodent tests.

**Non-Radioactive Assays**

Current non-radioactive assays are generally based on flow cytometry. These methods have a good correlation with the $^{51}$Cr release assay (2,3) and also have the advantage of being able to identify lytic events before they are detectable with the $^{51}$Cr release assay. The target cells are labeled with a fluorescent dye to render discrimination between the populations of effector cells and target cells possible. To identify permeabilized target cells a fluorescent DNA stain, which labels only cells with compromised plasma membranes, is added. This technique enables a clear separation between live and dead target cells (Figure 1). The endpoint shows a direct proportion of killed target cells as percentage of specific cytolysis calculated by the formula:

$$\left(\frac{\% \text{ dead targets in the sample} - \% \text{ spontaneously dead targets}}{100 - \% \text{ spontaneously dead targets}}\right) \times 100$$

**Natural Killer Cell Assay. Figure 1** Natural killer (NK) cell activity in Wistar rat peripheral blood measured by flow cytometric analysis of CFSE-labeled and PI-labeled YAC-1 targets. Results are shown as FL1(CFSE)/FL3(PI) dot plots gated on target cells using CellQuest (Becton Dickinson, USA) analysis software. Viable targets are given in R2. Non-viable targets are given in R3. Cells were cultivated for 18 hours. A: Control without effector (E) cells shows 1.7% spontaneously dead target cells (T) in R3. B: An E:T ratio of 50:1 and addition of IL-2 shows an NK cell activity of 63.7% dead target cells in R3.
The choice of target cell labeling is crucial for this test to be successful. The target cell membranes are labeled with a primary fluorescent dye, e.g. F-18, FITC, PKH-2, PKH-26, or CFSE (5-(6)-carboxy-fluorescein succinimidyl ester). After co-incubation with effector cells, a secondary fluorescent DNA-binding dye (e.g. propidium iodide (PI) or TO-PRO-3 iodide) is added for discrimination of dead target cells. Leakage of the primary dye can sometimes contribute to cross-contamination of cells other than the targets as described with fluorescein or PKH-2. To avoid cross-contamination, CFSE is preferable as a primary dye. It is added to the unlabeled targets as non-fluorescent 5-(6)-carboxy-fluorescein diacetate, succinimidyl ester and will diffuse passively into the cells. Within the membrane of viable cells esterases remove the acetyl moieties leaving CFSE that binds to proteins and is well retained within the cell. CFSE is non-toxic, does not interfere with cell function, is suitable for long-term NK assays (16–20 h) which are needed to render reading of NK activity in certain animal species possible, and it has the fluorescent excitation and emission characteristics of fluorescein and is suitable for use in combination with the DNA binding dye, PI. CFSE and PI can be readily distinguished by flow cytometry, using the FL1- and FL3-channel respectively, omitting the need for compensation of spectral overlap. Targets and effectors are co-incubated for 18 hours (rat) or 4 hours (human) followed by flow cytometric analysis. The assay can be performed with or without addition of interleukin(IL)-2. Approximately $2.5 \times 10^5$ effector cells are needed per sample using an E : T ratio of 100 : 1. The low amount of effectors required indulges the use of peripheral blood in rodent studies as well as repeated testing of the same animal (3). Another non-radioactive assay used for measuring NK cell activity is the lactate dehydrogenase (LDH) release assay. Since this assay cannot discriminate between LDH released from dead effector cells or dead target cells it has limited use, especially in long-term cultures.

**Pros and Cons**

One of the advantages of the flow cytometric assay compared to the classical $^{51}$Cr release assay is the low amount of effector cells needed. This minimal requirement of effectors allows for the use of peripheral blood as an effector source and also for repeated testing of the same animal. A summary of advantages and disadvantages with the two methods are shown in Table 1.

**Predictivity**

Host resistance models are ultimate tests in risk assessment of consequences that a decrease in any immune parameter, such as NK cell activity, may have for resistance against infection or tumor cells. When changes in NK cell activity ($^{51}$Cr release), in mice, are compared to changes in host resistance the concordance is 73% (4). Consequently, NK cell function is regarded as a valuable predictor of experimentally induced immunotoxicity. Still, NK assays are generally more sensitive than host resistance assays. In mice, a decrease in NK activity of ≥ 50%–60% is required before a decrease in specific host resistance to tumors can be detected (1).

NK cell activity measured as described in this section covers only one NK-mediated pathway of cytotoxicity: secretory/necrotic killing by perforin/granzyme. Mechanisms for elimination of, for example, solid tumours are not covered. To make a complete determination of NK cell function per se, additional mechanism-related tests are needed (5). Also, for each chemical tested specific attention should be paid to which NK cell compartment is most suitable for isolation of effector cells (3). The test substance’s route of exposure could be crucial considering this choice.

**Relevance to Humans**

Various drugs have shown to affect NK cell function in animal studies or after in vitro exposure of human cells (3). However, the clinical relevance of these data needs further evaluation. Impaired cytotoxic NK cell activity has been associated with acute virus infections, multiple sclerosis, AIDS, systemic lupus erythematosus, and leukemia. Evidences for a correlation between reduced NK cell activity and cancer in man are scarce but medium and high cytotoxic activity of peripheral blood is associated with reduced cancer risk, while low activity is associated with increased cancer risk.

**Regulatory Environment**

The European EMEA guidance on immunotoxicity testing of new drugs for human use (CPMP/SWP/1042/99; October 2000) encourages NK cell function as an immunotoxicity parameter in standard non-clinical repeat-dose toxicity studies. Testing of NK cell activity is suggested as a first-hand alternative to another functional assay (Primary antibody response to a T-cell dependent antigen) with the aim of avoiding the use of satellite animal groups required by the latter. It should be noted that none of these functional assays are meant to act as stand-alone tests and that the results always should be interpreted along with other toxicological findings and general health status of the animal. The FDA guidance for industry on immunotoxicology evaluation of investigational new drugs (October 2002) includes determination of NK function as a follow-up test if warranted based on findings in standard repeat-dose toxicology studies.
Natural Killer Cells

**Synonyms**

NK cells

**Definition**

Natural killer (NK) cells are lymphoid-derived cellular components of the innate immune system. These cells are part of the first line of defense that function to contain viral infections as well as the spread of tumor cells. The measurement of NK cell activity has recently become a critical assay used to identify potentially immunotoxic chemicals.

**Characteristics**

NK cells are derived from the bone marrow and comprise approximately 5%–15% of human peripheral blood lymphocytes (1). They lack surface expression of antigen-specific receptors, as well as many of the surface molecules such as CD3, CD4, CD8 or...
CD19, that are typically found on B and T lymphocytes. NK cells contain large cytoplasmic granules that aid in the killing of pathogen-infected cells, tumor cells, and major histocompatibility complex (MHC) class I disparate cells. However, recognition and killing of target cells by NK cells is intricately regulated by a combination of activating and inhibitory receptors.

The activation of NK cells to kill infected cells is accomplished following the ligation of several distinct cell surface receptors (Table 1).

A heterogeneous family of NK cell-specific immunoglobulin-like molecules has recently been identified. They are known as natural cytotoxicity receptors, and they activate NK cells when bound to as yet undefined ligands. This receptor family includes NKp30, NKp44 and NKp46 (2,3). In addition, NKG2D (a member of the lectin superfamily) also activates NK cells following stimulation by the MHC class I polypeptide-related sequence A/B (MICA/MICB) and UL16-binding proteins (ULBPs). In another somewhat distinct mechanism, CD16 (an immunoglobulin G Fc receptor (FcγRIIIa) that is expressed on NK cells and phagocytic cells) can provide activating signals to NK cells following the cross-linking of bound IgG (2). The primary outcome of CD16-mediated NK cell activation is the generation of antibody-dependent cell-mediated cytotoxicity (ADCC). Alternatively, NK cells are activated by interferons (IFN-α and IFN-β) and cytokines such as the interleukins IL-2, IL-12, IL-15, IL-18 and tumor necrosis factor TNF-α (3). Stimulation of NK cells by these soluble mediators can increase their activity up to 100-fold as measured by the NK cell assay (1). Furthermore, activation by these cytokines leads to increased proliferation, trafficking, and cytotoxicity by NK cells, as well as inducing them to secrete copious amounts of IFN-γ—crucial in controlling various infections. NK cells can also secrete other effector cytokines including TNF, granulocyte macrophage colony stimulating factor (GM-CSF), IL-5, IL-10 and IL-13 (2).

To regulate the activation of NK cells and prevent the killing of normal host cells, a collection of MHC class I-specific inhibitory receptors exist (Table 1). In humans, NK cell inhibitory receptors include members of the killer-cell immunoglobulin-like receptor (KIR) family that bind to MHC class I molecules, and the CD94–NKG2A heterodimer that recognizes non-polymorphic human leukocyte antigen HLA-E molecules (2,3). In mice, the inhibitory receptors on NK cells that recognize non-self MHC class alleles are called Ly49 (1). Rodents also express the CD94–NKG2 receptor. Signaling via the NK inhibitory receptors suppresses the killing activity of NK cells and serves to protect healthy cells that express self MHC class I molecules.

Following the activation of NK cells (in the absence of inhibitory signals), tumor or virus-infected target cells can be killed by two separate mechanisms. The first mechanism of NK cell-mediated killing is identical to

### Natural Killer Cells. Table 1 Receptors involved in the regulation of natural killer cell function

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Species</th>
<th>Ligand</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Activating receptors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NKG2D</td>
<td>Human</td>
<td>MICA/MICB, ULBP1, -2, -3</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>Rae-1, H60</td>
</tr>
<tr>
<td>NKp30</td>
<td>Human</td>
<td>Unknown</td>
</tr>
<tr>
<td>NKp44</td>
<td>Human</td>
<td>Unknown</td>
</tr>
<tr>
<td>NKp46</td>
<td>Human</td>
<td>Unknown</td>
</tr>
<tr>
<td>CD16</td>
<td>Human/mouse</td>
<td>IgG</td>
</tr>
<tr>
<td><strong>Inhibitory receptors</strong></td>
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<td></td>
</tr>
<tr>
<td>KIR2DL1</td>
<td>Human</td>
<td>HLA-C</td>
</tr>
<tr>
<td>KIR2DL2/3</td>
<td>Human</td>
<td>HLA-C</td>
</tr>
<tr>
<td>KIR3DL1</td>
<td>Human</td>
<td>HLA-B</td>
</tr>
<tr>
<td>KIR3DL2</td>
<td>Human</td>
<td>HLA-A</td>
</tr>
<tr>
<td>Ly49</td>
<td>Mouse</td>
<td>H-2K, H-2D</td>
</tr>
<tr>
<td>CD94-NKG2A</td>
<td>Human</td>
<td>HLA-E</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>Qa-1</td>
</tr>
</tbody>
</table>

HLA, human leukocyte antigen; KIR, killer cell immunoglobulin-like receptor; MHC, major histocompatibility complex; MICA/ MICB, MHC class I polypeptide-related sequence A/B; ULBP, UL16 binding protein.
that used by cytotoxic T lymphocytes (CTL); cytotoxic granules are secreted onto the cell surface of bound target cells where they penetrate the membrane and induce apoptosis (1). The second mechanism also involves the induction of apoptosis in target cells although via a non-secretory pathway. This form of cytotoxicity results from the engagement of death receptor pathways such as CD178 (FasL) on the surface of NK cells and CD95 (Fas) on target cells (2). Other TNF family members including TNF and TNF-related apoptosis-inducing ligands (TRAIL) also mediate NK cell killing via their respective receptors on tumor cells.

**Preclinical Relevance**

NK cell activity is affected by a number of drugs and environmental chemicals. Although chemical-induced changes in NK cell activity have been demonstrated to possess a high concordance with altered host resistance, it remains to be determined if these alterations in immune function are physiologically relevant (5). The investigation of NK cells and NK cell activity following exposure to drugs and environmental chemicals is regulated by special guidelines. NK cell activity is commonly employed as an initial screening test for use in non-clinical, repeated-dose immunotoxicity studies.

**Relevance to Humans**

Because of the importance of the innate immune system in the early response to infection, loss of NK cells or their functions following exposure to drugs and xenobiotics could possibly lead to an increased susceptibility to disease. This point is illustrated in a number of known NK cell deficiencies and associated infectious susceptibilities that exist in humans (and also rodents). For examples, individuals harboring alterations in CD16 gene expression via several polymorphisms are at increased risk of developing frequent upper respiratory viral infections, recurrent bacterial infections, and even autoimmune disorders (4). In people suffering from functional NK cell deficiency (FNKD), NK cells are present but they lack one or more NK cell functions (4). These individuals are highly susceptible to several pathogens, including Epstein-Barr virus (EBV) and herpes simplex virus (HSV) due to a lack of NK cell cytotoxicity. Another well-characterized immunodeficiency resulting from aberrations in NK cell cytotoxic effector mechanisms is Chediak-Higashi syndrome (CHS). NK cells in patients with CHS are defective in spontaneous cytotoxicity and ADCC, and possess abnormal morphology (4). The molecular pathogenesis of this disease involves mutations in the *LYST* gene which normally regulates lysosomal trafficking. CHS patients have recurrent respiratory bacterial infections, suscep-

**Regulatory Environment**

Recently, the European Agency for the Evaluation of Medicinal Products recommended that testing of NK cell activity be included in the initial immunotoxicity screening of medicines for human use (5). This test is to accompany other non-functional parameters of the immune system such as hematology, lymphoid organ weights, histopathology, bone marrow cellularity, and lymphocyte subset analysis. In addition, the US Food and Drug Administration (FDA) insists on the inclusion of NK cell activity testing for the safety evaluation of pharmaceuticals (5). However, this regulatory agency recommends that NK cell evaluation be considered a Tier II test, which is only to be performed if adverse effects are detected in non-functional Tier I assays as described above. NK cell activity is also currently being considered by the Japanese Ministry of Health, Labor and Welfare to be included as an assay in drug safety evaluation (5).

**References**


**Natural Killer 51Cr Release Assay**

- Natural Killer Cell Assay
**Natural Killer (NK) Cell**

Natural killer (NK) cells are non-T and non-B lymphocytes that can kill some types of tumor cells, virus-infected cells, and cells infected with other types of intracellular pathogens. NK cells have innate mechanisms for target cell recognition, as well as an antibody-dependent mechanism (ADCC). The innate mechanism is mediated by a family of stimulatory and inhibitory receptors that are encoded by the NK gene complex.

**Cell-Mediated Lysis**

**Respiratory Infections**

**Cytotoxicity Assays**

**Flow Cytometry**

**Dermatological Infections**

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**Natural Killer (NK) Cell Assay**

The chromium release assay involves the ex vivo killing of a suitable cell line which is labeled with radioactive chromium, typically using splenocytes or peripheral blood mononuclear cells from animals dosed with the test article.

**Canine Immune System**

**Flow Cytometry**

**Dermatological Infections**

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**Negative Selection**

The process in the thymus that leads to the elimination of unreactive or autoreactive thymocytes.

**Thymus: A Mediator of T Cell Development and Potential Target of Toxicological Agents**

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**Neoantigen-Forming Chemicals**

Chemicals that can capable of eliciting an immune response not necessarily have to act as haptens, but they may also alter normal self proteins, e.g. by modifying their expression, by oxidising single amino acid side chains, or by complex formation, in such a way that other peptides or larger amounts of certain peptides of the altered protein are presented by antigen-presenting cells. Since these peptides may not have been ‘seen’ by the immune system before (i.e. they are new antigens or neoantigens), no T-cell tolerance towards them is established and, therefore, an immune response against them may be initiated.

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**Neonatal Immune Response**

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**Synonyms**

Newborn immune function, neonatal tolerance

**Definition**

The immune responses of neonatal mammals, including man, remain immature at birth. Neonatal immune responses are characterized by failure to respond to polysaccharide antigens, delay in antibody production, absence of isotype switching, and unusual susceptibility to tolerance induction following antigen (Ag) exposure. This period of neonatal immunodeficiency persists for up to 6 weeks in rodents and several years in humans.

**Characteristics**

**Architecture of the neonatal immune system.** The immune system of mammals is formed embryonically in a progression of tissue microenvironments that develop sequentially. Prior to birth, precursors for immunocompetent cells are formed and expand in embryonic tissues, however, mature cell function can not be demonstrated prior to birth. Neonatal immunity differs from adult immunity in anatomical features and functional outcomes.

**Neonatal antibody production.** The spleen microenvironment in the newborn differs from that of adults. Splenic primary follicles of B lymphocytes are not observed until 2 weeks after birth in mice. Secondary follicles or germinal centers are detected in the spleen of mice until 4 weeks of age. The formation of splenic germinal centers is known to be essential for cell interactions necessary for T-dependent activation of B lymphocytes and subsequent antibody formation in response to T-dependent antigens (1). In spleens of human neonates, germinal centers are not detected until several months following birth.

B lymphocytes from neonates produce lesser amounts of antibodies than do cells from adult animals. Most importantly, neonates do not produce antibodies when challenged with polysaccharide antigens. This is has been particularly noted when mice were challenged with lipopolysaccharide-rich (LPS) antigens extracted...
from bacteria (2). It has also been determined that while stimulation of adult B cells by antigen binding to the B cell receptor (BCR) confers activation and cell proliferation, neonatal B cells often undergo apoptosis following antigen binding (3). This ease of tolerance induction in B cells may have importance in establishing tolerance to self-antigens early in life. Neonatal B cells do not effectively antibody isotype switching from IgM to other Ig classes, including IgG and IgA for some time after birth. Because of neonatal B cells, IgA is not found in mucosal secretions until about 4 weeks of age in mice and at about 2 years in humans.

Transference of maternal antibody protection. Newborns acquire protective immunoglobulins (Ig) passively through the mother from placental transfer before birth and postnatal lactation. These antibodies protect newborns from foreign antigen exposure during the period of neonatal immunodeficiency; however, they also blunt active immunity in the neonate by eradicating antigen before it can stimulate newly formed B cells in the newborn. Maternal gammaglobulin antibodies have a half-life of approximately 90 days and decrease rapidly following cessation of breast feeding.

Neonatal cell mediate immune responses. CD4+ T helper cells and CD8+ cytotoxic T cells are present in secondary lymphoid tissues of neonates, however, cell mediated immune response are damped in neonates following engagement of the T cell receptor (TCR) (4). This observation is likely related to differences in cytokine secretion by these cells. Stimulated T cells from newborns produce less of the cytokines Interleukin-2 (IL-2), granulocyte-macrophage colony stimulating factor (GM-CSF), interferon-γ (INF-γ) and interleukin-5 (IL-5) when compared to cytokine production by adult T cells (3). This difference in cytokine production also results in reduced antibody responses to T cell dependent (TD) antigens and decreased immune responsiveness to antigen exposure in general (5). Some studies have also suggested that neonatal T cells require greater levels of co-stimulatory molecule interaction involving CD2, CD3, and CD28, in addition to TCR engagement than do adult T cells.

Preclinical Relevance
Studies of immune responsiveness to antigen challenge in neonatal mice and humans differ dramatically from that demonstrated for adults. Design of vaccination schedules for developing adaptive immunity to pathogenic organisms requires careful attention to this the period of neonatal immunodeficiency and the timing of maturation of the immune response to specific antigen challenges.

Relevance to Humans
Human infants do not respond to polysaccharide or T-dependent antigens some time after birth. This period of neonatal immunodeficiency has to be considered in both design of vaccination schedules and investigation of altered immune mechanisms related to immunodeficiency. It is also important to consider the role of maternal antibodies in responses to pathogenic organisms or vaccination to antigens during the period of neonatal immunity.

Regulatory Environment
There is considerable interest in detecting immunotoxicity following chemical exposure in animal models and in exposed human populations. Developing regulatory testing methods for immunotoxic compounds must consider both the unusual vulnerability and the altered responsiveness of the immune system during the perinatal period.

References

**Neonatal Immune Response. Table 1 Maturation of the Immune Response**

<table>
<thead>
<tr>
<th>Immune Function</th>
<th>Rodent</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antibody-Mediated Immune Responses</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TI-1 Responses</td>
<td>at birth</td>
<td>at birth</td>
</tr>
<tr>
<td>TI-2 Responses</td>
<td>1 month</td>
<td>1 year</td>
</tr>
<tr>
<td>TD Isotype Switching</td>
<td>1 month</td>
<td>&gt; 1 year</td>
</tr>
<tr>
<td>TD Affinity Maturation</td>
<td>1 month</td>
<td>&gt; 1 year</td>
</tr>
<tr>
<td>TD Ab Heterogeneity</td>
<td>1 month</td>
<td>2 years</td>
</tr>
<tr>
<td><strong>Cell-Mediated Immune Responses</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tolerance Induction</td>
<td>3 days</td>
<td>&gt; 7 days</td>
</tr>
<tr>
<td>Cytokine Production</td>
<td>1 month</td>
<td>&gt; 1 month</td>
</tr>
</tbody>
</table>

Neonatal Tolerance

Neonatal Immune Response

Neural Tube Defect

Defects where the vertebra or back of the skull fail to close, allowing spinal cord or brain—and associated tissues—to protrude. In humans such defects involving spinal cord and brain are also referred to as spina bifida and exencephaly, respectively.

Neonates, Immune Protection Against

Neuroendocrine Response

Neuroimmunological Evaluation of Antiglobulin (Coombs) Test

Neurotransmitter

A chemical which acts as a messenger between cells in the brain and nervous system; it transmits impulses across the gap from a neuron to another neuron, a muscle or a gland.

Neurons

The morphologic unit of the nervous system, consisting of the nerve cell body and its various processes, the dendrites and the axis cylinder process or neuraxon; the axis cylinder process of a nerve cell; a neuraxon.

Neutrophil

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Synonyms
Polymorphonuclear neutrophil, granulocyte

Definitions
Neutrophils are terminally differentiated cells, rich in cytoplasmic granules, containing a lobulated chromatin-dense nucleus with no nucleolus. Four types of cytosolic granules have been characterized.

Characteristics
Neutrophils represent 50%–60% of the total circulating leukocytes in the nontraumatized human. In rodents, neutrophils represent a much smaller percentage of the circulating leukocytes. These cells constitute the first line of defense against infectious agents and other invading substances. Once an inflammatory response is initiated, neutrophils are often the first cells recruited to the site of infection or injury. Neutrophil microbiocidal processes consist of the formation of a combination of reactive oxygen (and possibly nitrogen) species and various hydrolytic enzymes and polypeptides. Neutrophils mature in the bone marrow prior to being released into the circulation, where they spend only 4–10 hours before marginating and entering tissue pools, where they survive 1–2 days in the absence of survival factors such as granulocyte-colony stimulating factor (G-CSF). Senescent neutrophils are thought to undergo apoptosis prior to removal by macrophages. Apoptosis is a means to clear to neutrophil without release of their cytotoxic contents and may play a role in the termination of the acute phase of an inflammatory response. Cells of the circulating and marginating pools can exchange with each other. Morphological maturation stages of neutrophils include myeloblasts, promyelocytes, myelocytes, metamyelocytes, bands, and finally segmented neutrophils.
Surface expression of various antigens during the stages of neutrophil development are well characterized. For example, CD16b, CD 35 and CD10 appear with neutrophilic maturation and CD49b and CD64 expression is down regulated during maturation (1).

Under normal circumstances, neutrophils are produced in the human bone marrow at the rate of $10^{11}$ cells per day (2). This process is controlled by two CSFs (G-CSF and granulocyte macrophage-CSF (GM-CSF)) that direct the production and differentiation of bone marrow progenitor cells. During states of stress (such as trauma) and infection, the rate of neutrophil differentiation can increase as much as 10-fold.

During an inflammatory response, chemotactic factors are generated which signal the recruitment of additional neutrophils to the site of injury and/or infection. Under normal conditions, neutrophils roll along microvascular walls via low-affinity interactions of selectins with endothelial carbohydrate ligands. During an inflammatory response, $\beta_2$-integrins and high affinity binding to intracellular adhesion molecules is activated on the activated endothelial cells signaling the first step in transmigration to the site of inflammation.

**Preclinical Relevance**

Neutrophils are the first line of defense in the control of infection and are intimately involved in the initiation of inflammatory responses in response to trauma. Alterations in neutrophil function can potentially affect host resistance to multiple invading agents. Further, increased neutrophil function or prolonged residence at the site of injury can result in tissue damage. Therefore, neutrophils have the potential to mediate or be involved in many of the immunopathological events contributing to disease.

**Neutrophil Function in Infection Control**

Polymorphonuclear neutrophils generate nonspecific immune responses capable of controlling bacterial invasion that also risk injuring or destroying normal, viable tissue. While many factors modify this response, the interaction between adhesion molecules on the vascular endothelium and ligands on circulating neutrophils lead to neutrophil attachment, priming, and activation. These interactions can affect the subsequent neutrophil function and resulting inflammatory responses. Various events, such as hemorrhagic shock, sepsis and tissue injury result in rapid upregulation and increased expression of adhesion molecules. Primed neutrophils are extremely sensitive to activating agents that render the cells capable of producing high levels of reactive oxygen metabolites (ROI). Together with phagocytosis and proteolytic enzymes, the generation of ROI by neutrophils is of central importance to the innate host defense to bacterial infection. In fact, chronic granulomatous disease is a rare genetic disorder characterized by severe, recurrent infections due to the inability of neutrophils and macrophages to mount an adequate respiratory burst to kill invading bacteria.

**Neutrophil Recruitment after Trauma**

Trauma, including major surgery, stimulates a cascade of events that mediate the inflammatory response. Activation of the complement system and of neutrophils is an early response to surgical trauma. Agents that are stimulated by surgical trauma and influence neutrophil production, apoptosis and function include interleukins IL-1, IL-6, IL-8, tumor necrosis factor (TNF), CSFs and bioactive lipids, such as platelet activating factor. In fact, several citations have reported a relationship between the degree of surgical trauma and the release of inflammatory mediators. Further, surgical stress (in particular postoperatively) is associated with a marked increase in the level of circulating catecholamines. The $\alpha$-adrenergic catecholamines markedly enhance neutrophil numbers. Induction of anesthesia alone was shown to induce a slight increase in number of circulating neutrophils. With the initiation of surgery, the number of circulating neutrophils increased up to 4.5 times during the surgery itself. The amount and duration of increase may be related to the degree of trauma induced during the surgical procedure. This may be explained by the observation that major, but not minor, surgery correlates with a reduction in neutrophil apoptosis. Peripheral blood neutrophil function has also been shown to be modulated by surgical trauma and induction of anesthesia. For example, it was shown that neutrophil chemotaxis was reduced up to 36% simply by the induction of anesthesia, but this inhibition was reversed by the surgical procedure. Further, neutrophil respiratory burst activity, enzyme content, microbioidal killing and surface proteins have also been shown to be modulated by surgical trauma.

**Relevance to Humans**

In human beings, there are severe consequences to either a reduction in neutrophil number or function: increased incidence, severity and duration of bacterial and fungal infections, or increased neutrophil function (i.e. tissue damage and destruction leading to immunopathological changes).

**Consequences of Neutropenia**

Hematopoietic cells destined to become mature neutrophils move through three cellular compartments before they enter the blood. Neutropenia results from disorders of stem cells, defects in the processes of proliferation and differentiation and abnormalities in the distribution and turnover of blood cells (3,4). Neutropenia is defined as a decrease in the absolute neu-
trophil count (ANC) to below normal levels. The ANC can vary widely in healthy individuals due to exercise, emotional state and circadian rhythm. In general, the level of neutropenia is defined as mild, moderate and severe based upon the risk of pyogenic infections when that level of neutropenia is sustained over 2–3 months.

Neutropenic patients are usually infected by organisms of their endogenous flora, the resident bacteria of the mouth, oropharynx, gastrointestinal tract and skin. Overall, gingivitis and mouth ulcerations are the most common problems resulting from neutropenia. In general, patients with severe chronic neutropenia have fewer serious infections than those with the same degree of neutropenia resulting from cancer chemotherapy. Chemotherapy-induced neutropenia broadly affects host-defense function, including the barrier role of the mucosal cells in the mouth and gastrointestinal tract.

Neutrophils in Inflammatory Disease States

Although the neutrophil response is designed to restrict the damage to the region surrounding the invading organism, collateral damage to surrounding tissues often occurs during the control of the invading organism. There is evidence from clinical studies that exaggerated recruitment and activation of neutrophils are linked to several inflammatory disorders including asthma, and chronic bronchitis (5). This may be due to the release of bioactive products that can actively contribute to the pathogenic process. Neutrophils can release cytokines that perpetuate neutrophil recruitment (e.g. tumor necrosis factor), along with proteolytic enzymes (e.g. elastase), bioactive lipids (e.g. prostaglandins) and ROI. While all of these products are necessary to the proper function of the neutrophil in the clearance of bacterial and fungal infections, they contribute to tissue damage when the number of activated neutrophils at a site is prolonged.

Regulatory Environment

Neutrophils are a granulocytic cell of hematopoietic origin that is central to the innate immune response to bacterial and fungal infection. Neutropenia results in an increased susceptibility to disorders they are meant to protect against; however, leukocytosis or prolonged neutrophilia can contribute to inflammatory disease pathogenesis.

Neutrophil numbers are evaluated in preclinical safety evaluation during hematological evaluation, and assessment of bone marrow. Further, immunopathological consequences of increased neutrophil number, residence or function is assessed during histopathological evaluation of multiple tissues. These studies are conducted as a routine in the evaluation of the safety of a new agent. Further evaluation of neutrophil function can occur during specialized tests to evaluate potential immunotoxicology. Initially, alterations in host resistance may indicate an effect on neutrophil number or function. If indicated, studies of neutrophil function may be conducted as a follow-up to alterations indicative of possible change in host resistance models.

References


Neutrophils

Short-lived bone-marrow-derived non replicating blood leukocytes, with a distinctive condensed chromatin (a nucleus of three to five lobes), that are specialized for phagocytosis and the killing of microbes.

- Opsonization and Phagocytosis
- Respiratory Infections

Newborn Immune Function

- Neonatal Immune Response

NF-kappa B (NFkB)

This is nuclear factor of kappa light-chain enhancer in B cells. It occurs in numerous cells and is activated by a range of stimuli. Both inappropriate activation and suppression are associated with adverse conditions, e.g. inflammatory processes, inappropriate immune cell development. After activation, NFkB translocates to the cell nucleus, where it binds to DNA and regulates transcription.

- Lymphocyte Proliferation
**NHL**

- Lymphoma

**Niacin**

Nicotinic acid; Vitamin B₃.

- Serotonin

**Nitro-PAH**

- Polycyclic Aromatic Hydrocarbons (PAHs) and the Immune System

**Nitrophenyl-Chicken gamma Globulin**

A T-dependent antigen prepared from chicken gamma globulin. Used primarily as a T-dependent antigen for ELISA determination.

- Plaque Versus ELISA Assays. Evaluation of Humoral Immune Responses to T-Dependent Antigens

**NK Cells**

- Natural Killer Cells

**NK Gene Complex (NKC)**

The NK gene complex (NKC) consists of a large family of cell surface receptors that act to either inhibit or stimulate the recognition and lysis of cells by natural killer (NK) cells.

- Cell-Mediated Lysis

**NK Cell Killing**

- Cell-Mediated Lysis

**No Observable Adverse Effect Level (NoAEL)**

The “no observable adverse effect level” is the xenobiotics dose at which no undesirable (toxic) effects are seen in animal studies.

- Knockout, Genetic

**Nodus lymphaticus**

- Lymph Nodes

**Non-Caseating Granuloma**

Non-caseating granuloma is a discrete nodule of multinucleated giant cells and lymphocytes that encapsulate persistent antigen to minimize tissue damage.

- Chronic Beryllium Disease

**Non-Hodgkin’s Lymphoma**

- Lymphoma

**Non-Obese Diabetic Mouse (NOD)**

Murine model for Insulin-dependent diabetes mellitus. Genetic alteration of the specific MHC alleles in this model has helped to elucidate the role of gene polymorphisms in disease susceptibility.

- Autoimmune Disease, Animal Models

**Non-Radioactive Flow Cytometric Analysis of NK Cell Cytotoxicity**

- Natural Killer Cell Assay

**Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)**

A class of compounds which inhibit the cyclooxygenase activity of prostaglandin H synthase (COX), thereby preventing prostaglandin synthesis and allevi-
ating prostaglandin-induced symptoms such as pain, fever, and inflammation.

**Prostaglandins**

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**Nonhuman Primates, Immunotoxicity Assessment of Pharmaceuticals in**

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**Synonyms**  
Preclinical immunotoxicity evaluation in the nonhuman primate.

**Definition**  
Assessment of immunotoxicity is a prerequisite for all new drugs in Europe. In the US only several classes of drugs require immunotoxicity evaluation. For conventional pharmaceuticals, rodent models commonly yield satisfactory results concerning effects on cells, organs, and functions of the immune system. The targeted design of more sophisticated drugs, especially biotechnology-derived substances, requires investigations in animal models that are more closely related to the human. Nonhuman primates are the species of choice to detect highly specific immunotoxic side effects, to avoid immunogenicity issues associated with rodent models and to discriminate toxicity from efficacy of immunomodulatory drugs (1).

**Characteristics**  
Unlike immunotoxicity studies in rodents, no standardized tests and protocols are generally available for nonhuman primates as yet. However, the rodent tests can be transferred in principle. An exception is the host resistance assay. Since changes in the virulence of the challenge-pathogen can lead to variability in the assay, comparatively large group sizes are required which can pose a limitation upon nonhuman primate usage (see below). Specialized facilities are needed to maintain infected animals and separate them from the main colony. This is usually more demanding and expensive for nonhuman primates than for rodents. Using death as a test endpoint is obviated by ethical considerations.

The choice of species and assay should always depend on the precise question and/or test article. Cynomolgus monkeys and rhesus monkeys (*Macaca fascicularis* and *M. mulatta*) and the common marmoset (*Callithrix jacchus*) are the nonhuman primate species used most commonly in toxicology testing. Due to the inherent properties of immunomodulatory substances (e.g. cross-reacting cytokines) immunotoxicity can show signs of either immunosuppression (potentially associated with reduced resistance to infections or cancer) or immunostimulation (potentially associated with autoimmunity or hypersensitivity). Therefore, immunotoxicity evaluation in nonhuman primates should not be confined to merely testing immunosuppression, as has been done until recently. The design of (immuno)toxicity studies in nonhuman primates generally invokes specific considerations. For ethical and cost reasons the group sizes in these studies are usually kept comparatively small. This, and the fact that no inbred strains of nonhuman primates are available, can impair statistical power for detection of test article-related effects. Interindividual variation of parameters of interest can be higher for nonhuman primates than for humans or inbred rodents. Therefore, it is advisable to include individual comparisons of baseline (pre-dose) evaluation.

Evidently, more test article is needed for immunotoxicity studies in nonhuman primates as compared to rodents. If the amount of test article is limited, the use of the smaller marmoset monkey should be considered.

**Preclinical Relevance**  
Preclinical relevance is generally the major justification for using nonhuman primates in toxicological studies. For the evaluation of biologics, monkeys should be chosen as test system for a variety of reasons:

- Closer homology for amino-acid sequence reduces the risk and probability for lack of biological activity. For example, the dose-limiting clinical toxicity of recombinant human interferon (IFN)-γ could only be reproduced in the nonhuman primate model.
- Closer homology for amino-acid sequence reduces the risk and probability of antibody formation and subsequent neutralizing antibody activity and absent or decreased exposure activity.
- Many antibodies for human immunological assays cross-react with nonhuman primate molecules and the same assay kits can be used for preclinical and clinical evaluation.

A rodent homolog or a transgenic rodent model represent the current alternatives for the use of nonhuman primate models. However, due to the interspecies differences in the immune systems and the multiple functions and feedback loops of immunomodulatory molecules, a species most closely resembling the human systems should always be used during evaluation of potential immunotoxicology.
Relevance to Humans

When considering preclinical studies to evaluate the influence of biologics, immunomodulatory drugs or vaccines on the immune system, species most closely related to humans should be considered (2). Among the primates, hominoid monkeys (e.g. chimpanzees) are most closely related to humans with evolutionary separation 6–14 × 10^6 years ago. Ethical concerns, governmental impact and costs are the reasons for not using these animals in immunotoxicity studies. Old World monkeys (e.g. baboons or macaques) are at the second closest phylogenetic distance (25 × 10^6 years) followed by New World monkeys (e.g. marmosets, 40 × 10^6 years). Old World monkeys, as well as hominoid monkeys, for example, have major histocompatibility complex (MHC) molecules which much resemble their human counterparts. Although the repertoire of MHC alleles might differ, the organization and expression pattern is comparable. New World monkeys in contrast show a condensed or smaller MHC as compared to humans. The strong relevance of Old World monkey models to humans is evident from the fact that the majority of the anti-human CD antibodies cross-react with the corresponding molecules in Old World monkeys but to a much lesser extent with New World monkeys. On the other hand, the marmoset immune system seems to share some—albeit not yet fully understood—similarities to humans. For example, the course of pertussis infection in marmosets mimics the infection of children more precisely than in Old World monkeys.

For the evaluation of reproductive or developmental immunotoxicity, macaques are the species of choice. There are close physiologic similarities with regard to the endocrine control of female and male gonadal functions, pregnancy, and prenatal development (3,4). The postnatal development of the immune system of macaques shares similarity to that of the developing human, for example in the levels of immunoglobulins and maturation of immune cells.

Regulatory Environment

Several guidelines for immunotoxicology evaluation have been issued. A new drug must be examined for immunotoxicity in the relevant species (for review see House and Thomas (5)). Nonhuman primates are frequently the relevant animal model for immunotoxicology evaluation of biologics. Initially, descriptive parameters such as hematology, clinical chemistry, weight and microscopy of lymphoid organs and bone-marrow cellularity are required. The European Agency for the Evaluation of Medical Products (EMEA) also encourages immunophenotyping, and recommends a functional test for natural killer cell activity at that stage (CPMP/SWP/0142/99: Note for Guidance on Repeated Dose Toxicity: Appendix B, Guidance on Immunotoxicity, October 2000). Immunotoxicologic findings strongly suggest additional follow-up studies to investigate the nature and mechanism of the immunotoxic effects. The choice of assays for these studies should be scientifically motivated. The Center for Drug Evaluation and Research (CDER) within the US Food and Drug Administration recommends immunophenotyping and functional assays such as the natural killer cell activity or antibody response investigation (CDER: Guidance

### Table 1

<table>
<thead>
<tr>
<th>Function measured</th>
<th>Assay</th>
<th>Source</th>
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</thead>
<tbody>
<tr>
<td>Structural integrity</td>
<td>Hematology/clinical chemistry</td>
<td>Ex vivo</td>
</tr>
<tr>
<td></td>
<td>Histopathology</td>
<td>Necropsy</td>
</tr>
<tr>
<td></td>
<td>Immunohistochemistry</td>
<td>Necropsy</td>
</tr>
<tr>
<td></td>
<td>Flow cytometry (Immunophenotyping)</td>
<td>Ex vivo</td>
</tr>
<tr>
<td>B-cell function</td>
<td>Antibody production (e.g anti-KLH response)</td>
<td>In vivo/ex vivo</td>
</tr>
<tr>
<td></td>
<td>Mitogenesis</td>
<td>Ex vivo</td>
</tr>
<tr>
<td>T-cell function</td>
<td>Delayed-type hypersensitivity</td>
<td>In vivo</td>
</tr>
<tr>
<td></td>
<td>Cytokine analysis</td>
<td>Ex vivo/in vitro</td>
</tr>
<tr>
<td></td>
<td>Mitogenesis or activation markers</td>
<td>Ex vivo/in vitro</td>
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<tr>
<td>Natural immunity</td>
<td>Natural killer cell function</td>
<td>Ex vivo</td>
</tr>
<tr>
<td></td>
<td>Macrophage/neutrophil function</td>
<td>Ex vivo</td>
</tr>
</tbody>
</table>

KLH, keyhole limpet hemocyanin.

* Immunotoxicity tests available for nonhuman primates (modified from House & Thomas (5)). Other tests might as well be performed, since no standardized tests are available.

References

Nonparametric Statistics
A set of statistical techniques which make no assumptions regarding the underlying distribution of the data. ► Statistics in Immunotoxicology

Nonsteroidal Antiinflammatory Drugs
► Anti-inflammatory (Nonsteroidal) Drugs

Norepinephrine (Noradrenaline)
A catecholamine hormone secreted by the adrenal medulla and a neurotransmitter released by postganglionic nerve cells. It is released predominantly in response to hypotension and stress. It acts on α- and β1-adrenergic receptors and is a powerful vasopressor. ► Stress and the Immune System

Northern
The transfer of RNA molecules from an agarose gel to a membrane by capillarity or an electric field. The immobilized RNA can be detected at high sensitivity by hybridization to a sequence specific probe. ► Southern and Northern Blotting

Nucleic Acid Blotting
► Southern and Northern Blotting

Nucleic Acid Vaccines
► DNA Vaccines

Nosocomial
Pertaining to or originating in a hospital. ► Streptococcus Infection and Immunity

NSAID-Activated Gene (NAG-1)
A divergent member of the transforming growth factor (TGF)-β superfamily which is induced by non-steroidal anti-inflammatory drugs (NSAIDs) and appears to play a role in the pro-apoptotic and anti-tumorigenic properties of NSAIDs. ► Prostaglandins

NSAIDs
► Anti-inflammatory (Nonsteroidal) Drugs

Nuclear Factor κB (NFκB)
Nuclear factor κB (NFκB) is a transcription factor central to a major signaling pathway induced by a plethora of stimuli including inflammatory agents such as TNF-α. NFκB is normally sequestered in the cytoplasm by interaction with inhibitor of κB proteins (IκB). Activation of the NFκB-inducing kinase cascade results in phosphorylation-dependent ubiquitination of IκB leading to proteolytic degradation and freeing of NFκB. NFκB then translocates to the nucleus via a nuclear localization signal sequence and binds to NFκB-response elements in the promoters of numerous genes involved in cell survival, proliferation, differentiation, and inflammation resulting in upregulation of gene expression. ► Tumor Necrosis Factor-α

Prostaglandins

Streptococcus Infection and Immunity

Tumor Necrosis Factor-α

DNA Vaccines
Nude Mouse

This a genetically athymic mouse (e.g. devoid of T-cells and cell-mediated immune capability) which also carries a closely linked gene involved in hair production.

Graft-Versus-Host Reaction

Null Mutant Mouse

Knockout, Genetic

Nurse Cell

Epithelial cell in the outer cortex of the thymus; enclose multiple thymocytes and add to the thymocyte maturation process.

Thymus

Nutrition and the Immune System

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Synonyms
Immunonutrition.

Definition
Many nutrients in the diet play important roles in maintaining optimal immune function. Nutrient deficiency is associated with an impaired immune response, particularly in cell-mediated immunity, phagocytic function, antibody response, and the complement system. Worldwide, malnutrition is the most common cause of immunodeficiency. Substances such as amino acids, nucleotides, probiotics and fatty acids can be added to standard nutritional support solutions, and the use of such formulations is known as immunonutrition.

Characteristics
There are three main sites in the immune system that can be targeted by specific nutrients: 1) the mucosal barrier; 2) cell mediated immunity; 3) local or systemic inflammation. The following substances are examples of nutrients that can be used to target one or more of these three important components of immunity.

Glutamine
Glutamine is an essential nutrient for immune cells both as a primary fuel and as a nitrogen donor for nucleotide precursor synthesis. Laboratory data have demonstrated numerous effects of glutamine on immune cells: macrophage phagocytosis in vitro declines when glutamine concentrations are lowered, glutamine supplementation significantly enhances phytohaemagglutinin (PHA)-stimulated lymphocyte proliferation and there is evidence of improved bactericidal function of neutrophils following glutamine supplementation in vitro. In addition, glutamine has been reported to restore mucosal immunoglobulin A, to enhance upper respiratory tract immunity, and enhance bacterial clearance in peritonitis.

Arginine
Arginine is considered a non-essential amino acid although endogenous supplies are reduced during trauma and sepsis. Importantly, arginine (via the arginine deaminase pathway) is a unique substrate for the production of the biological effector molecule nitric oxide and it is via this molecule that arginine is thought to mediate many of its immunomodulatory effects. Clinical evidence suggests that arginine enhances the depressed immune systems of patients suffering from injury, surgery, malnutrition or sepsis, by acting on cellular defense mechanisms. Arginine supplementation has many effects on immune cells such as enhanced lymphocyte and monocyte proliferation, enhanced T helper cell formation and activation of macrophage cytotoxicity.

Nucleotides
In the case of adequate protein intake, de novo synthesis is the main source of nucleotides with glutamine being the major nucleotide donor. During episodes of infection following trauma or injury, the demand for nucleotides is increased to facilitate the synthetic capacity of immune cells. Decreased nucleotide availability has many effects on immune cells such as impaired T cell function, weakened natural killer cell activity, suppressed lymphocyte proliferation, reduced phagocytosis, and impaired clearance of pathogens.

Probiotics
The gut flora are believed to confer immunological protection on the host by creating a barrier against pathogenic bacteria. Antibiotic use and disease can lead to disruption of this barrier leaving the host gut susceptible to pathogens. It is now believed that this barrier can be maintained by dietary supplements...
called “probiotics” which are live “desirable” bacteria. In addition to creating a barrier effect, some probiotic bacteria produce proteins which inhibit the growth of pathogens or the probiotic bacteria themselves compete with pathogens for nutrients. There is also some evidence these beneficial bacteria can enhance gut immune responses: rat and mouse studies reveal that orally administered lactic acid bacteria increase numbers of T lymphocytes, CD4+ cells and antibody secreting cells, enhance lymphocyte proliferation, natural killer activity, cytokine production and phagocytic activity in macrophages.

**n-3 Polyunsaturated Fatty Acids**

There is much interest in the anti-inflammatory effects of n-3 polyunsaturated fatty acids (PUFA). Dietary n-6 and n-3 PUFA modulate the lipid content of membrane phospholipids which in turn affects eicosanoid production. A diet rich in n-6 PUFA favors synthesis of eicosanoids derived from the arachidonic acid (AA) precursor, whereas a diet rich in n-3 PUFA shifts the balance of eicosanoids synthesized to favor those derived from docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). DHA and EPA, which are found in fish oil, can decrease the production of the proinflammatory cytokines, decrease lymphocyte proliferation, and suppress autoimmune disease, although the exact mechanism is unclear.

**Relevance to Humans**

There are many clinical applications of immunonutrition. Various enteral formulas are available containing immune-modulating substances such as glutamine, arginine and n-3 PUFA, and the clinical benefits of such formulas have been shown in post-operative and critically ill patients. Clinical trials with these formulas show clear evidence for reduced incidence of infections, reduced duration of ventilation, and shortened hospital stays. Several trials have shown immune-enhancing effects of dietary probiotic supplementation.

**References**
