REVIEW, SYNTHESIS, AND APPLICATION OF INFORMATION ON THE HUMAN LYMPHATIC SYSTEM TO RADIATION DOSIMETRY FOR CHRONIC LYMPHOCYTIC LEUKEMIA

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ABSTRACT

While chronic lymphocytic leukemia (CLL) has been traditionally considered a non-radiogenic disease, recent studies have suggested that a relationship between CLL and exposure to ionizing radiation could, in fact, be real (although probably weak). Cells of origin for CLL appear to be mature B lymphocytes which could have been transformed into cancerous cells potentially anywhere within the lymphatic system. To estimate the risk of CLL from an exposure to radiation, one would need to calculate a radiation dose to the population of CLL precursor cells. Such a calculation is not trivial because CLL precursor cells can be present in different compartments of the lymphatic system located throughout the body, and these compartments can receive substantially different doses. The situation is further complicated by the fact that the CLL precursor cells may travel within and even outside the lymphatic system, and their inventories in various compartments of the body may change significantly with age, gender, health status and other factors.

This paper analyzes several approaches to determine a meaningful radiation dose for the assessment of radiological risk of CLL. We concluded that a practical, but not necessarily ideal, solution is a dose estimated as an average across all body compartments containing CLL precursors, weighted by the inventory of CLL precursors in each compartment. For this purpose, we collected relevant information about the lymphatic system, about the distribution of lymphocytes in general, and B lymphocytes and CLL precursors in particular. We found that lymph nodes *in toto*, spleen, intestinal mucosa and red bone marrow contain the largest percentage of B lymphocytes and, probably, of CLL precursors. Although the current available information is rather limited, it is possible to define a meaningful dose using concepts of probabilistic uncertainty analysis. That is, probability distributions were used to describe the uncertainty in the inventories of CLL precursors in each compartment of interest. These uncertainties were propagated using Monte-Carlo simulations to determine a probability distribution for a weighted dose based on the relative inventory either of B cells or of CLL precursors in different body compartments. The resulting probability distribution describes the uncertainty in the inventory-weighted average dose, and can be used to define a credibility range containing the true but unknown dose. This probability distribution can be used as an input in any probabilistic risk assessment for CLL in adults exposed to radiation.

PART I REVIEW AND SYNTHESIS OF INFORMATION ON LYMPHATIC SYSTEM

1. INTRODUCTION

Although ionizing radiation is a well-known cause of leukemia, most papers on the topic exclude chronic lymphocytic leukemia (CLL) from consideration because it is thought to be well established that CLL is not radiogenic. For example, the U.S. Energy Employees Occupational Illness Compensation Program (USEEOICP) does not currently recognize claims for CLL resulting from radiation exposure. However, recent information suggests that the case against CLL as a radiogenic disease may not be as clear-cut as once thought (Hamblin 2008; also see Silver et al. 2007). In addition, under the atomic veterans compensation program in the U. S., the Veteran's Administration (VA) *does consider claims for CLL* occurring in military personnel who were exposed to radiation during tests or uses of atmospheric nuclear weapons.

If it is determined that CLL should be considered a potentially radiogenic disease, it will not be a simple matter to link a given exposure to radiation to the risk of developing the disease because of current uncertainty about the appropriate target organ or tissue to use in radiological risk assessment. The target organ or tissue for purposes of radiological risk assessment is the irradiated site in which a normal stem or stem-like cell undergoes transformation to a cancerous clone.

Claims from nuclear workers who have developed cancer are evaluated using outputs from models that relate radiation doses to a specific site (the target organ or tissue) to the probability of causation of a radiogenic cancer at that site (Kocher et al. 2008). For most cancers, the identification of the target organ or tissue is straightforward. For example, the risk of developing liver or thyroid cancer is estimated from the radiation dose to the entire liver or thyroid gland. For other cancer types (e.g., oral cavity, stomach, or colon), the dose of interest is that to the tissues within these organs that contain the cells which lead to cancer (e.g., to the lining of the stomach).

The leukemias *per se*—as distinguished from diseases such as CLL and hairy cell leukemia which are forms of non-Hodgkins lymphoma (NHL)—are monoclonal diseases that arise from

hematopoietic stem and progenitor cells located in the red bone marrow.[1] Thus, the red bone marrow is considered both the sole target organ for radiogenic leukemias and the site in which the pathological effects of the leukemic clones develop.

However, current information indicates that the cells of origin of NHL are *mature* B lymphocytes, i.e., cells that could have been transformed to cancerous clones *outside* the bone marrow and, in the case of CLL, potentially anywhere within the circulatory or lymphatic systems (e.g., because of normal lymphocyte movements and recirculation). For CLL, a considerable body of information suggests that the precursor cell is also antigen-experienced and could be a memory cell (Klein et al. 2001; Chiorazzi et al. 2005; Caligaris-Cappio and Ghia 2007; Ghia et al. 2007; Chiorazzi 2007; Linet et al. 2007; Zent 2007).[2, 3] Thus, the precursor for CLL is most probably a mature, antigen-experienced lymphocyte of currently uncertain lineage (among the known subsets of normal B cells) and of unknown location in lymphoid tissues when it was transformed to a CLL cell (by radiation or some other stimulus) (Klein et al. 2001; Rosenwald et al. 2001; Chiorazzi et al. 2005; Caligaris-Cappio and Ghia 2007; Ghia et al. 2007; Chiorazzi 2007).[4] Thus, the risk of developing CLL from radiation exposure cannot be estimated using a conventional "target organ" approach.[5]

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[1] Chronic lymphocytic leukemia appears etiologically and clinically to be a lymphoma and thus differs from the other forms of leukemia (Harris et al. 1999; NCI 2008a). It is a disorder of morphologically mature but less immunologically mature lymphocytes, manifested by progressive accumulation of these cells in the blood, bone marrow, and lymphoid tissues (Chiorazzi et al. 2005; NCI 2008b). It is considered a form of NHL by the U.S. National Cancer Institute (NCI 2008a) and the World Health Organization (Harris et al. 1999). Small lymphocytic lymphoma and CLL are characterized as a single disease entity in their classifications of hematopoietic and lymphoid diseases.

[2] Other potential precursors for CLL: circulating V-preB$^+$L$^+$ cells; transitional B cells; potential human equivalents of the murine B-1 cell; CD5$^+$ follicular mantle B cells; and marginal zone B cells (many of which are memory cells) (Chiorazzi et al. 2005; Hervé et al. 2005; Chiorazzi 2007).

[3] CLL is characterized by progressive accumulation of functionally incompetent, long-lived small mature monoclonal B lymphocytes with a typical phenotype (CD19$^+$, CD5$^+$, CD23$^+$) and low B-cell receptor expression resulting from low surface immunoglobulin and CD79b expression (Abramenko et al. 2008).

[4] CLL cases can be divided into two groups, depending on the degree of somatic hypermutation of the immunoglobulin V_H genes. Cases from the "unmutated" group (having V_H gene sequences with <2% differences from the germ line cells) have a much more aggressive disease course than those from the mutated group.

[5] A risk model based largely on the incidence of NHL is currently being used by the VA to assess the probability of causation (PC) of CLL occurring in U. S. military personnel. The PC of CLL occurring in these individuals is estimated using the highest of the reconstructed doses to one of three organs: red bone marrow, spleen, or thymus. The rationale is that since CLL is now considered to be a form of NHL, red bone marrow is not the sole target organ. In contrast, the sole target organ used for assessing the PC of *all B-cell lymphomas (including NHL)* resulting from internal exposures to nuclear workers under the US EEOICPA is the collection of thoracic lymph nodes (ORAU 2006). The first approach can be criticized because it includes the thymus which has few B cells but ignores the lymph nodes which have many. The second because the thoracic lymph nodes contain relatively few B lymphocytes and do not receive elevated doses from ingested radioactivity, and it does not include organs/tissues that do.

The purpose of this paper is to determine whether it is feasible to estimate a meaningful radiation dose for CLL for use in radiological risk assessment, given current uncertainties about the etiology of the disease, including but not limited to the nature of the CLL precursor and the site in which the precursor is transformed to a CLL clone.

2. BACKGROUND

Because radiation doses from internally deposited radionuclides and also from some types of external exposures can vary significantly with location in the body, B lymphocytes at different sites could receive markedly different doses. For example, following inhalation of insoluble forms of plutonium, B lymphocytes resident in the thoracic or extrathoracic lymph nodes could receive radiation doses several orders of magnitude greater than those to the spleen, intestinal mucosa, or red bone marrow (ICRP 2002b, 2006). In theory, information about the probability that a potential B-CLL precursor was resident in such a location along with an estimate of the length of time that it could have been exposed to radiation at that site could be used to estimate the radiation dose. However, estimating these parameters is problematic because of current uncertainties about the behavior and localization of the half-dozen types of B cells that are currently considered to be potential CLL precursors.

Thus, estimation of a meaningful radiation dose for a disease such as CLL appears to require a probabilistic approach to account for both the location and the movement of CLL precursor cells. A first-order approach to this problem might entail assigning probabilities to radiation doses measured or calculated at different locations in the body based solely on estimates of the relative numbers of potential CLL precursors at these locations, i.e., by assigning relative weights to each compartment while ignoring the effects of lymphocyte circulation and movements between sites. An assumption inherent in such an approach is that the lymphatic system can be treated as a system in dynamic equilibrium (but see later discussion in Sects. 5.1 and 5.3). At a minimum, such an approach would require a comprehensive set of information on the inventories and distributions of potential B-lymphocyte precursors of CLL in all compartments (i.e., organs, tissues, and fluid reservoirs) of the human body.

Unfortunately, there is currently no single comprehensive and authoritative source of information on the numbers and distribution of lymphocytes, let alone B lymphocytes and the

subsets relevant to CLL, in compartments of the human body for use in radiation dosimetry and risk assessment. The information on the lymphatic system in the ICRP's (2002a) set of basic anatomical and physiological data for use in radiological protection is inadequate to the purpose, largely outdated, and, in several important cases, simply erroneous.[6]

As Pabst et al. (2008) have recently pointed out, our information on the lymphocyte distribution in the body is still quite limited, not only for humans, but also for laboratory animals such as mice and rats. Although extrapolations from measurements in laboratory animals have been used by various authors to fill in the gaps in human data (Trepel 1974; Brandtzaeg et al. 1989; Westermann and Pabst 1992; Blum and Pabst 2007; Ganusov and De Boer 2007), uncertainties in the animal data (e.g., for the respiratory mucosa) limit the effectiveness of such extrapolations (see, e.g., Pabst et al. 2008). This is a critical point because it needs to be understood clearly at the outset that there is currently no complete set of data on the inventories and distributions of lymphocytes in compartments of the human body that is based *exclusively* on human data.

The ability to define the location of B cells is complicated because the lymphatic system is spread out within the body, and some components are quite diffuse (e.g., lymphocytes and isolated lymphoid follicles in the respiratory and intestinal mucosa). While lymphoid organs such as spleen, thymus, or bone marrow have reasonably well-defined locations, discrete accumulations of lymphocytes and lymphoid follicles[7] in the form of lymph nodes are widely distributed spatially (Fig.I.1). In addition, there are important age-related changes that affect the activity and sizes of the lymph nodes, with one result being that their sizes may not reflect their immunological significance or the numbers of lymphocytes they contain (MacDonald 2008a; Pabst et al. 2008).

This report is organized into two parts. Part I attempts to provide a compilation and synthesis of existing information on inventories and distribution of B lymphocytes in compartments of the human body to support efforts in radiological risk assessment for potentially radiogenic diseases

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[6] For example, the length of the human small intestine is underestimated by a factor of 2 (~3 m vs 6.6 m; cf Pabst et al. 2008). Errors such as this appear to have been introduced in revising the 1975 predecessor to the ICRP report.

[7] The main functional components of the lymph nodes and other secondary lymphoid organs are the follicles (sometimes called nodular lymphoid tissue), which are collections of regular structures (polarized and organized regions) containing both B and T lymphocytes. The compartments in which CLL proliferates are termed pseudofollicles, vaguely nodular areas without mantles that are observed in lymph nodes and bone marrow (Stevenson and Caligaris-Cappio 2004). The pathological features of the lymph node in which CLL develops are those of a small lymphocytic lymphoma (Chiorazzi et al. 2005).
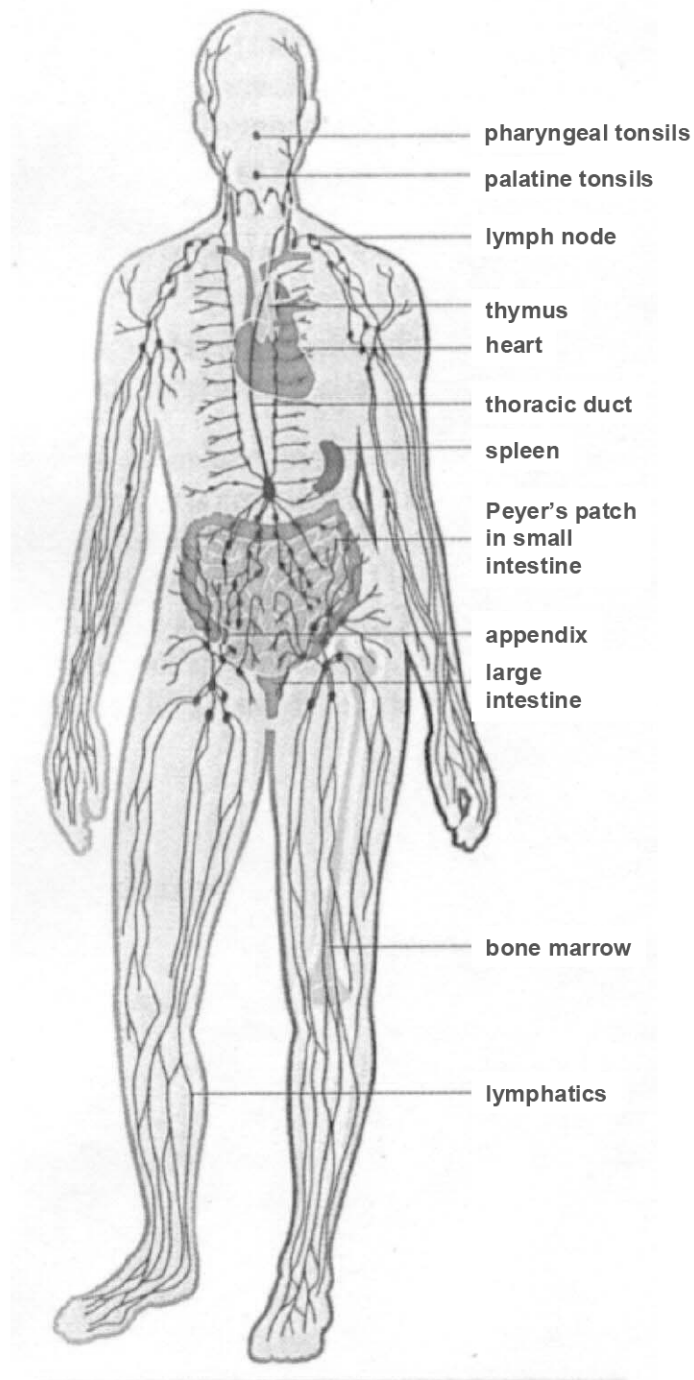
Fig.I.1. The lymphoid organs. Source: Adapted from DeFranco et al. (2007).

such as CLL. Several options for applying this information to estimation of a meaningful radiation dose for CLL are examined in Part II of this report.

3. APPROACH

We performed a literature search in an attempt to assemble a comprehensive set of information on inventories and distributions of B lymphocytes consistent with the stated need. We quickly discovered that published estimates of inventories of B lymphocytes or potential B-lymphocyte precursors for CLL (as opposed to inventories of lymphocytes as a group) in compartments of the human body were unavailable. In addition, information on regional distributions of lymph nodes had not been compiled and summarized in a manner suitable for use in radiation dosimetry. Thus, we had to both evaluate and integrate the available information before we could apply it for our purpose. This section describes the types of information available about inventories and distributions of lymphocytes and how this information was analyzed to derive compartment-specific weights based on relative sizes of B-lymphocyte pools to be used in estimating a weighted average radiation dose for assessment of the risk of developing radiogenic CLL.

3.1 Principal Sources of Information

The information we ultimately used can be separated into the following categories, covering information on:

- total number of lymphocytes of all types [B-, T-, and natural killer (NK) cells] in the human body;

- numbers of lymphocytes (all types combined) in a given organ, tissue, or other compartment in the body, both inside and outside the lymphatic system (e.g., Trepel 1974; Brandtzaeg et al. 1989; Blum and Pabst 2007; Pabst et al. 2008);

- anatomy and physiology of the lymphatic system, including
  - graphic and numerical information for estimating the total numbers and regional distribution (i.e., head, neck, upper limbs, thorax, abdomen, pelvis, and lower limbs) of the lymph nodes, which collectively have been thought to contain the largest single fraction of the inventory of lymphocytes in the human body (e.g., Gray 2000; Agur and Dalley 2005, but see MacDonald 2008a and Pabst et al. 2008);

> ➢ descriptive and numerical information on the numbers of lymphoid follicles in different organs of the lymphatic system (e.g., in Klein and Horejsi 1997), which can be used to estimate the total numbers of lymphocytes in organs for which inventories have not been reported (i.e., by comparison with numbers of follicles reported for organs that do have inventory estimates);

- fraction of the pool of lymphocytes in a given organ, tissue, or other compartment represented by B lymphocytes (e.g., from Westermann and Pabst 1992); and, to a much more limited extent,

- fraction represented by specific subsets of B lymphocytes, with emphasis on potential B-CLL precursors, data for which is found in widely scattered publications in the literature.

A variety of calculations and extrapolations, many requiring application of Monte Carlo methods to propagate existing uncertainties in data, were performed to assemble a meaningful set of information on inventories and distributions of B cells.

3.2 Synthesis to Generate Inventories of B lymphocytes and CLL Precursors

Because of the nature of the information, a sequence of calculations was needed to convert information on inventories of lymphocytes in body compartments, first to inventories of B lymphocytes (Section 4.3) and then to inventories of potential B-cell precursors for CLL in these compartments (Section 4.5). This was done by applying information on the fractions of lymphocytes represented by B cells and potential CLL precursors in these compartments to estimates of total inventories of lymphocytes.

Some judgment was also required to generate compartment-specific inventories of B lymphocytes because there is not a one-to-one correspondence between compartments with lymphocyte inventory data and those with information on fractions of B lymphocytes (e.g., for information on intestinal and respiratory mucosa). In addition, in some cases only a single published value was available for the lymphocyte inventory or B-lymphocyte fraction in a body compartment.

Probability distribution functions (PDFs) were assigned to the number of lymphocytes and to the fraction representing B cells for each organ of interest based on (1) ranges of values found

in the literature, (2) ranges of values based on professional judgment, or (3) results of specific calculations (as described in Section 4.1.2). The ranges of values we used are thought to represent potential extremes (i.e., reasonable estimates of minima and maxima based on our review). Thus, unless otherwise noted, these ranges were used to define the 1st and 99th percentiles of the assigned PDFs, which are intended to reflect uncertainty due to the current lack of knowledge about the true value.

The uncertainties in the published estimates of total lymphocyte pools in human body compartments appear to be on the order of +100%, −50% (or a factor of 2)—and, in some cases more (Pabst et al. 2008; MacDonald 2008a). In cases where only a single published value was available, we assigned an uncertainty range equal to +100%, −50% of that value.

The uncertainties in the published estimates of the B-lymphocyte fractions in specific body compartments were on the order of ±50%. Thus, in those cases where only a single published value was available, we assigned an estimate of uncertainty equal to ±50% of that value.

Lognormal distributions were used to estimate uncertainties in the numbers of lymphocytes in all body compartments other than tonsils (for which a uniform distribution was used) and the lamina propria of the intestinal mucosa (for which a Weibull distribution was used because it provided a better representation of the data). Normal distributions were used to quantify uncertainties in the fractions of total lymphocytes represented by B cells for most body compartments. Weibull distributions were used for lymph nodes, vermiform appendix and greater omentum; uniform distributions for thymus and skin; and a triangular distribution for spleen. The shape of the distribution was selected according to the nature and quality of the available data (e.g., a uniform distribution means that the true value is believed to be anywhere in the provided range with equal probability, while lognormal, normal, Weibull and triangular distributions assume that there is a better chance that the true value is around the provided central value, and that the provided central value and range can be fitted by a distribution with the selected shape.) Uniform, normal and triangular distributions were typically applied when the state of knowledge indicated small uncertainty. Log-transformed distributions reflect uncertainties that were expressed as a multiplier (e.g. a factor of 2) around a median.

Monte Carlo methods have been used to propagate the uncertainties in the numbers of lymphocytes and in the fractions of B lymphocytes in individual body compartments. That is, the number of B lymphocytes in a given organ was obtained by multiplying the PDF for the

number of lymphocytes in that compartment by the PDF for the fraction of the pool of lymphocytes represented by B cells (expressed as percentages in our tabulated information). The ranges of values for the number of B lymphocytes in individual body compartments are 95% credibility (rather than confidence) intervals obtained using Latin Hypercube sampling (LHS) and a sample size of 2000. These ranges are termed credibility intervals because they rely on judgment-based ranges for the number of lymphocytes in that compartment or for the fraction of the pool of lymphocytes in the compartment represented by B cells.

We also estimated the percentages of the total number of B lymphocytes in the body located in specific compartments. These values were obtained from the Monte Carlo uncertainty propagation algorithm as follows. In the first Monte Carlo iteration, one value of the number of lymphocytes in each compartment and one value of the fraction represented by B lymphocytes were sampled from the input distributions for all compartments of interest. The number of lymphocytes and the fraction of B lymphocytes were multiplied to obtain one estimate of the number of B lymphocytes for each compartment of interest. An estimate of the percentage of the total B lymphocytes in the body present in any given organ was obtained by dividing the number of B lymphocytes in the compartment of interest by the total number of B lymphocytes (obtained as a sum of the number of B lymphocytes across all compartments). The operation was repeated for each of the 2000 Latin Hypercube samples. The resulting uncertainty ranges represent 95% credibility intervals, rather than statistical confidence intervals, because they are based, in part, on judgment regarding estimates of the ranges of lymphocyte inventories and the fraction of the inventories represented by B cells.

A similar operation was performed to obtain estimates of the percentages of potential CLL precursors in the body present in a specific compartment. That is, the number of lymphocytes in a compartment was combined with the fraction of the pool of lymphocytes represented by B cells and with an uncertain fraction of B cells that could be CLL precursors in that same compartment (see text in Section 4.5 for details).

Details about the available data, underlying assumptions, assigned PDFs, flow of calculations, and resulting inventories are discussed in the next section. The application of this information (i.e., extension of the calculations, also using Monte Carlo methods) to estimate a meaningful radiation dose for CLL is discussed in Part II of this report.

4. PRESENTATION OF RESULTS FROM REVIEW AND SYNTHESIS

Inventories of lymphocytes (all types combined) in body compartments and the fractions that are B lymphocytes are presented in Sections 4.1 and 4.2, respectively. The synthesis of this information to produce estimates of inventories of B lymphocytes in body compartments is summarized in Section 4.3. Our estimates of the numbers and locations of lymph nodes are presented and discussed in Sect. 4.4. Current information on fractional distributions of potential B-lymphocyte precursors for CLL is reviewed in Section 4.5. The latter is used in combination with inventories of B lymphocytes presented in Section 4.3 to generate estimates of inventories of potential B-lymphocyte precursors for CLL in body compartments.

4.1 Inventories of Lymphocytes in Body Compartments

Estimated inventories of lymphocytes of all types (B, T, and NK cells) in individual body compartment are given in Table I.1. These inventories were based on a combination of mainly human data and limited animal (mostly rat) data. The animal data has been used to cover gaps in human data (see, e.g., Trepel 1974; Brandtzaeg et al. 1989; Westermann and Pabst 1992; Blum and Pabst 2007; Ganusov and De Boer 2007; Pabst et al. 2008). We have also obtained independent and/or more recent data for the pools of lymphocytes in blood, intestinal mucosa, lung, liver, lymph, and Peyer's patches in humans. The estimated number of diffuse lymphocytes located in the respiratory mucosa as a whole and in a catchall "Others" category (see Table I.1) is based in part on extrapolation of data from animals (Trepel 1974; Brandtzaeg et al. 1989; Blum and Pabst 2007). The "Others" category includes a variety of minor contributors to the overall lymphocyte pool: muscle, lymph, skin, body cavity fluids, etc.

The synthesis of current published information on lymphocyte inventories in Table I.1 includes estimates of the numbers of lymphocytes in lymph nodes *in toto*, spleen, Peyer's patches, thymus, bone marrow, tonsils, peripheral blood, intestinal mucosa (lamina propria), respiratory mucosa (lung parenchyma), and "Others" (lymph, skin, liver, vermiform appendix, interstitial fluids of the body cavity, cerebrospinal fluid, and residual soft tissue). Our residual soft tissue pool is intended to represent muscle, mucosa of the urogenital tract, greater omentum, and some of the "Others" constituents listed individually: lymph and body cavity fluids.

Table I.1. Estimated distribution of lymphocytes in the human body

Compartments of the human lymphatic system		Number of lymphocytes [$\times 10^9$]	Reference or derivation
Discrete lymphoid organs/tissues			
Lymph nodes		190 (95−380)[a, b]	Trepel (1974)
Spleen		75 (70; 80)[a, c]	Trepel (1974); Turesson (1976)
Peyer's patches		10 (6−20)[a]	Calculation[d]
Thymus		50 (25−100)[a, b]	Trepel (1974)
Red bone marrow		50 (25−100)[a, b]	Trepel (1974)
Tonsils		0.8 (0.2−2)[a]	Extrapolation[e]
Subtotal for discrete organs/tissues		**380 (290−540)**[f]	Sum of the above
Other lymphocyte-containing organs, tissues, or compartments			
Blood		**10 (5−25)**[a, g]	Trepel (1974); Abbas and Lichtman (2003); Alberts et al. (2002)
Intestinal mucosa (lamina propria)		**90 (30−180)**[a, b, h]	MacDonald (2008a)
Respiratory tract mucosa			
	Alveolar space	Not available	
	Epithelium	Not available	
	Interstitium	Not available	
	Lung parenchyma	4−10[a]	Holt et al. (1986)
Subtotal for Respiratory Mucosa		**30 (15−60)**[b, i]	Trepel (1974)
Others			
	Lymph	0.4 (0.2−0.8)[a, b]	Engeset et al. (1974)
	Skin	13 (6.5−26)[a, b]	Bos et al. (1987)
	Liver	6 (2; 10)[a, b]	Hata et al. (1990); Blum & Pabst (2007)[j]
	Vermiform appendix	0.2 (0.1−0.4)[a]	Extrapolation[k]
	Interstitial fluids of body cavity	Not available	
	Cerebrospinal fluid	0.0006[a]	Cashman et al. (1982)
	Residual soft tissue[l]	20 (10−40)[b]	Calculation[m]
Subtotal for Others		**40 (22−72)**[f, n]	Blum & Pabst (2007)
Grand total		**560 (440−730)**[f, o]	Overall summation obtained using Monte Carlo methods

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Footnotes for Table I.1

[a] Based on <u>human</u> data.

[b] Range was assumed to be -50%, $+100\%$ of central value.

[c] The central estimate is the arithmetic average of the lower value (reported by Trepel 1974 and Blum and Pabst 2007) and the higher value (from Turesson 1976) in the range.

[d] Values are the mean and 95% C.I. obtained by random sampling methods using data for the size distribution of Peyer's patches and numbers of follicles per patch reported by MacDonald (2008a), based on data reported by Cornes (1965), and the estimated number of lymphocytes in a follicle (10^6) provided by MacDonald (2008b). The upper 95% credibility limit has the same value as the lymphocyte inventory in Peyer's patches estimated by Trepel (1974) from a combination of rodent and human data.

[e] Estimated by taking ratio of numbers of lymphoid follicles in lingual tonsil [est. 100$-$300, based on 35$-$100 follicle-containing units reported by Klein and Horejsi (1997), and an estimate of 3 follicles per unit (see their Fig. 3.8b)], multiplying the value by factor of 4$\pm$2 to account for lymphoid tissue in the laryngeal, pharyngeal, palatine (2), and tubal (2) tonsils (see Fig. 3.7 and associated text in Klein and Horejsi 1997), and then multiplying the result by the estimated inventory in a follicle (10^6 lymphocytes) provided by MacDonald (2008b) to get an estimate of the total inventory in tonsils.

[f] Values rounded up to no more than two significant figures. Ranges estimated by Monte Carlo sampling methods as described in text.

[g] The central estimate is that reported by Trepel (1974). The range of values is that reported in Table 2-1 of Abbas and Lichtman (2003). A value of 15×10^9 is obtained from data for average blood volume and lymphocyte concentrations in adult humans reported in Table 22-1 of Alberts et al. (2002).

[h] Estimated by MacDonald (2008a) based on an estimate of 15,800 lymphoid follicles in the 1.4-m length of the large intestine reported by Langman and Rowland (1986), the mean length of the small intestine (6.6 m) reported by Cornes (1965), and the estimated number of lymphocytes in a follicle (10^6) from MacDonald (2008b). This estimate is much larger than the value of 30×10^9 lymphocytes reported by Trepel (1974). Trepel used the lymphocyte inventory (distributed outside Peyer's patches) in the intestinal mucosa that had been determined in rats, which was more than three times the number of blood lymphocytes in the same animals, to estimate the lymphocyte content of the human intestinal mucosa (i.e., by simply multiplying the blood inventory estimated in humans by a factor of three). Our range runs from the value estimated by Trepel (1974) to a value twice that of our central estimate of 90×10^9 lymphocytes.

[i] Based on the assumption by Trepel (1974) that the lymphocyte content of the respiratory mucosa was comparable to that obtained by extrapolating rat data for the intestinal mucosa to humans (see last two sentences in footnote g). However, Pabst et al. (2008) have pointed out that such an assumption is fallacious, because other mucosae have fewer lymphocytes than the intestinal mucosa.

[j] The central estimate is the arithmetic average of the lower value (estimated by us from data on the lymphocyte content of liver from Hata et al. 1990 and the mass of the liver in the adult human male from ICRP 2002a) and the higher value (from Blum and Pabst 2007) in the range.

[k] Estimated by taking the number of lymphoid follicles in the vermiform appendix (about 200) reported by Klein and Horejsi (1997) and multiplying by the number of lymphocytes per follicle (10^6) provided by MacDonald (2008b).

[l] Includes muscle, lymph, body cavity fluids, greater omentum, and mucosa of the urogenital tract.

[m] Estimated by subtracting the central estimates of lymphocyte pools in the skin, liver, tonsils, and appendix from the subtotal of 40×10^9 lymphocytes reported by Blum and Pabst (2007) (see footnote n).

[n] Blum and Pabst (2007) reported a value of 30×10^9 lymphocytes in "Other Tissues," which included tonsils, and a separate value of 10×10^9 lymphocytes for the liver. They did not explain why their estimate was four times larger than that originally estimated by Trepel (1974) for "Other Tissues," which included both tonsils and liver.

[o] For comparison, Trepel (1974) originally estimated a total of 460×10^9 lymphocytes, while Alberts et al. (2002) reported a total of 2000×10^9, but without giving a detailed breakout of lymphocyte numbers by organ, tissue, or other body compartment. Thus, there is potentially a large uncertainty in our values that may not have been accounted for.

Some authors (e.g., Trepel 1974; Blum and Pabst 2007) have included the liver and/or tonsils within this category.

4.1.1 Inventories Obtained Directly from Scientific Publications

The numbers of lymphocytes in the lymph nodes *in toto*, spleen, thymus, red bone marrow, blood, lung, and skin were obtained directly from the literature. The values for the lymph nodes, thymus, and red bone marrow were taken from the seminal report by Trepel (1974). The lymph nodes *in toto* appear to contain more than one third of the human lymphocyte inventory (Table I.1). Because the radiation doses to lymph nodes in specific regions (e.g., thoracic lymph nodes) can be much higher than those to nodes in other regions under certain conditions, we also performed a more extensive evaluation of the regional distribution of the lymph nodes (see Section 4.4).

The spleen was estimated to contain about 10−15% of the lymphocytes in the human body based on inventories reported by Trepel (1974), Turesson (1976), and Blum and Pabst (2007) (see Table I.1), but other sources suggest that the importance of the spleen might be greater, perhaps containing up to 25% of the body's lymphocytes (see Section 5.2.3). A 20-y-old healthy adult reportedly has 10,000−20,000 lymphoid follicles in his/her spleen (Klein and Horejsi 1997). However, the marginal zones of the spleen, located outside the follicular areas are also important sites for interaction with blood-borne antigens and subsequent activation of B cells. "Sessile" memory B cells, one of the prime candidates for the B-CLL precursor, are also reportedly found in this region (Kraal 1992).

4.1.2 Inventories Derived from Calculations

In several cases, inventory estimates given in Table I.1 were based on recent calculations or extrapolations made by us or others:

- **Peyer's Patches**—Values in Table I.1 are the mean and 95% C.I. obtained by random sampling methods using data for the size distribution of Peyer's patches and numbers of follicles per patch reported by MacDonald (2008a), based on data reported by Cornes (1965), and the estimated number of lymphocytes in a follicle (10^6) provided by MacDonald

(2008b). Coincidentally, our upper 95% credibility limit is the same as Trepel's (1974) estimate, which was based on a combination of rodent and human data.

- **Tonsils**—The inventory was estimated by taking the ratio of the numbers of lymphoid follicles in lingual tonsil [est. 100−300, based on 35−100 follicle-containing units reported by Klein and Horejsi (1997), and an estimate of 3 follicles per unit (see their Fig. 3.8b)], multiplying the value by factor of 4 ± 2 to account for lymphoid tissue in the laryngeal, pharyngeal, palatine (2), and tubal (2) tonsils (see Fig. 3.7 and associated text in Klein and Horejsi 1997), and then multiplying the result by the estimated inventory in a follicle (10^6 lymphocytes) provided by MacDonald (2008b) to get an estimate of the total inventory in tonsils. The basis for this estimate is quite crude, and it does not explicitly account for age-related changes in the activity of the tonsils (see later discussion in Section 5.1). However, because age-related changes are likely to reduce the significance (hence, the lymphocyte inventories) of the tonsils in adults, our estimate should be biased high. Since the estimated lymphocyte inventory in the tonsils is already <<1% of the total inventory in the human body (Table I.1), overestimating the inventory in these tissues should have a negligible effect on estimates of the overall inventory or the relative contribution of the tonsils.

- **Intestinal mucosa (lamina propria)**—The central estimate of 90×10^9 lymphocytes was derived by MacDonald (2008a) based on an estimate of 15,800 lymphoid follicles in the 1.4-m length of the large intestine reported by Langman and Rowland (1986), the mean length of the small intestine (6.6 m) reported by Cornes (1965), and the estimated number of lymphocytes in a follicle (10^6). [MacDonald assumed that the density of follicles in the small intestine was the same as that in the large intestine and rounded their estimate of the total number of follicles up to 100×10^9 to account for the increased mass of humans since the 1960s. We elected not to make this last adjustment.] Their estimate is much larger than the value of 30×10^9 lymphocytes reported by Trepel (1974). Trepel used the lymphocyte inventory (distributed outside Peyer's patches) in the intestinal mucosa that had been determined in rats, which was more than three times the number of blood lymphocytes in the same animals, to estimate the lymphocyte content of the human intestinal mucosa (i.e., by simply multiplying the blood inventory estimated in humans by a factor of three). Our range runs from the value estimated by Trepel (1974) to a value twice that of our central estimate

of 90×10^9 lymphocytes. Estimates of the inventory of lymphocytes in the intestinal mucosa that are much larger than the highest value in Table I.1 can be obtained. Such estimates and our rationale for not using them currently are discussed in Section 5.2.2.

- **Liver**—Our central estimate of 6×10^9 lymphocytes is the arithmetic average of the lower value (estimated by us from data on the lymphocyte content of liver from Hata et al. (1990) and the mass of the liver in the adult human male from ICRP (2002a) and the higher value in the range, taken from Blum and Pabst (2007).

- **Vermiform appendix**—Our central value of 0.2×10^9 lymphocytes was estimated by taking the number of lymphoid follicles in the vermiform appendix (about 200) reported by Klein and Horejsi (1997) and multiplying by the number of lymphocytes per follicle (10^6) provided by MacDonald (2008b). We assigned an uncertainty range equal to +100%, −50% of that value.

- **Residual soft tissues**—include muscle, lymph, body cavity fluids, greater omentum, and mucosa of the urogenital tract. Our central value of 20×10^9 lymphocytes was estimated by subtracting central estimates of the lymphocyte pools in the skin, liver, tonsils, and the appendix from the subtotal of 40×10^9 lymphocytes taken from Blum and Pabst (2007). It was also assigned an uncertainty range equal to +100%, −50% of that value. Blum and Pabst (2007) reported a value of 30×10^9 lymphocytes in "Other Tissues," which included tonsils, and a separate value of 10×10^9 lymphocytes for the liver. They did not explain why their estimate was four times larger than that originally estimated by Trepel (1974) for "Other Tissues," which included both tonsils and liver.

4.2 Fractions of Lymphocytes in Compartments That Are B Lymphocytes

The fractions of pools of lymphocytes in different compartments of the human body that are B cells was summarized by Westerman and Pabst (1992), and was based solely on human data (Table I.2). Numerous other studies and publications reporting fractions of lymphocytes that are B cells have been obtained and reviewed; the data collected from those studies has also been included where applicable and was used to define the uncertainty ranges in Table I.2 (see Notes in Table).

Table I.2. Estimated percentages of lymphocytes in human body compartments that are B lymphocytes[a]

Compartments of the human lymphatic system		% B lymphocytes (range)	Notes
Discrete lymphoid organs/tissues			
Lymph nodes		20 (15−30)	
Spleen		50 (20−60)	
Peyer's patches		40 (25−55)	
Thymus		0.55 (0.1−1)	
Red bone marrow		50 (20−80)	
Tonsils		50 (25−75)[b]	Range from Sesterhenn et al. (1977)
Other lymphocyte-containing organs, tissues, or compartments			
Blood		25 (10−40)	
Intestinal mucosa			
	Lamina propria	30 (15−45)[b]	
	Epithelium	1 (0.5−1.5)[b]	Estimated to represent 10% of lymphocyte inventory in mucosa outside Peyer's patches
Respiratory tract mucosa			
	Alveolar space	5 (2.5−7.5)[b]	Est. 10% of inventory in mucosa
	Epithelium	<1	
	Interstitium	Not available	
	Lung parenchyma	15 (5−25)	Est. 90% of inventory in mucosa
Others			
	Lymph-afferent	5 (2.5−7.5)[b]	
	Lymph-efferent	10 (5−15)[b]	
	Skin	<1 (0.1−1)	Range is hypothetical estimate
	Liver	10 (5−15)[b]	
	Vermiform appendix	20 (15−30)	Central value from Neiburger et al. (1976); range for lymph nodes assumed
	Peritoneal fluid	5 (2.5−7.5)[b]	Inventories/concentrations not available; assumed to be negligible
	Cerebrospinal fluid	5 (2.5−7.5)[b]	
	Greater omentum	70 (35−100)[b]	
	Residual soft tissue	7.5 (2.5−15)	Inventory/concentration not available; based on values reported for lymph

[a] Primary source: Westermann and Pabst (1992), except where noted.
[b] Range was assumed to be ±50% of central value.

B cells make up roughly half of the lymphocytes in the Peyer's patches, red bone marrow, spleen, tonsils, and greater omentum, while ~20% of the lymphocytes in the lymph nodes, blood, lung parenchyma, and vermiform appendix are B cells. The B-cell fraction in the remaining body compartments appears to be ≤10%, and in the thymus, skin, and mucosal epithelia the fractional representation of B lymphocytes is ≤1% (Table I.2).

4.3 Inventories of B Lymphocytes in Body Compartments

Our synthesis of information to develop estimates of the inventories of B lymphocytes in compartments of the adult human body is in Table I.3. The organs or tissues with the largest inventories of B lymphocytes are the lymph nodes *in toto*, spleen, red bone marrow, and the intestinal mucosa (Table I.3). [Although the lymphocyte pool in the thymus is comparable to that in red bone marrow, <1% are B lymphocytes. Similarly, the skin contains a significant number of lymphocytes (~10×10^9) but nearly all of them are T (or NK) cells (Westermann and Pabst 1992).]

Next in importance are the Peyer's patches, respiratory mucosa, peripheral blood, and residual soft tissue. Inventories of B cells in these sites are about an order of magnitude less than in the first four sites listed. Other sites have inventories that are two or more orders of magnitude less than the latter.

4.4 Numbers and Locations of Lymph Nodes

The lymph nodes *in toto* contain one of the largest pools of lymphocytes in general and of B cells in particular. They are distributed throughout the body in locations than can receive significantly different radiation doses. Thus, it is desirable to estimate the number lymph nodes at locations of differing radiological significance, with the intent to use this information as an indicator of the percentage of B lymphocytes at these locations relative to the total number of B lymphocytes in all lymph nodes taken together.

The number of lymph nodes in humans has been reported as about 500 (Trepel 1974) or 600-700 (ICRP 2002a). Using two different publications, we have independently estimated that humans have about 600−750 lymph nodes (Tables I.4 and I.5a−I.5f).

Table I.3. Estimated distribution of B lymphocytes in compartments of the human body[a]

Compartments of the human lymphatic system	Number of lymphocytes in compartment [x 10^9] (range)	% B lymphocytes (range)	B-lymphocyte inventory in compartment [x 10^9] (95% C.I.)[b]	% of total B lymphocytes in human body (95% C.I.)[b]
Discrete lymphoid organs/tissues				
Lymph nodes	190 (95–380)	20 (15–30)	38 (20–74)	28 (15–45)
Spleen	75 (70; 80)	50 (20–60)	33 (19–43)	23 (13–33)
Peyer's patches	10 (6–20)	40 (25–55)	4.3 (2.2–8.5)	3.1 (1.5–6.2)
Thymus	50 (25–100)	0.55 (0.1–1)	0.26 (0.05–0.66)	0.19 (0.04–0.50)
Red bone marrow	50 (25–100)	50 (20–80)	24 (11–51)	17 (8.0–32)
Tonsils	0.8 (0.2–2)	50 (25–75)	0.5 (0.11–1.2)	0.37 (0.08–0.87)
Other lymphocyte-containing organs, tissues, or compartments				
Blood	10 (5–25)	25 (10–40)	2.4 (0.95–6.1)	1.8 (0.60–4.5)
Intestinal mucosa				
Lamina propria	90 (30–180)	30 (15–45)		
Epithelium	10 (4–18)	1 (0.5–1.5)		
Subtotal: Intestinal Mucosa	**100 (40–180)**		26 (9.5–56)	19 (7–35)
Respiratory tract mucosa				
Alveolar space and epithelium	Est. 10% of inventory	3 (1–5)		
Interstitium	Est. <0.1%?	Not available		
Lung parenchyma	Est. 90% of inventory	15 (5–25)		
Subtotal: Respiratory Mucosa	**30 (15–60)**		4.1 (1.5–8.9)	2.9 (1.1–6.5)
Others				
Lymph	0.4 (0.2–0.8)	7.5 (2.5–15)	Included in residual	Included in residual
Skin	13 (6.5–26)	0.55 (0.1–1)	0.07 (0.01–0.17)	0.05 (0.01–0.12)
Liver	6 (2; 10)	10 (5–15)	0.58 (0.24–1.1)	0.41 (0.16–0.81)
Vermiform appendix	0.2 (0.1–0.4)	20 (15–30)	0.04 (0.02–0.08)	0.03 (0.02–0.04)
Peritoneal fluid	Not available	5 (2.5–7.5)		
Cerebrospinal fluid	0.0006	5 (2.5–7.5)	Included in residual	Included in residual
Greater omentum	Not available	70 (35–100)		
Residual soft tissue	20 (10–40)	7.5 (2.5–15)	1.5 (0.77–2.8)	1.0 (0.72–1.4)
Grand totals	**570 (440–740)[c]**		**140 (100–190)**	

[a] Based on data from Tables I.1 and I.2, unless otherwise indicated.
[b] Central estimates of B-cell inventories and of percentage distributions of B cells are medians and means, respectively.
[c] Central estimate is slightly higher than in Table I.1 because of adjustment to inventory in intestinal mucosa.

Table I.4. Regional distribution of lymph nodes in the human body and estimated percentage of total B-cell inventory in lymph nodes within region

LYMPH NODES	Number of nodes[a]			Percent of total B cells in lymph nodes within region (95% C.I.)[b]
Head and neck	120	–	160	21 (19, 24)
Upper limbs	60	–	70	10 (9, 11)
Thorax	100	–	130	17 (15, 20)
Abdomen	240	–	280	40 (37, 42)
Pelvis	35	–	45	6 (5, 7)
Lower limbs	35	–	45	6 (5, 7)
TOTAL	**600**	**–**	**750**	**100**

[a] Sources: Gray (2000); Agur and Dalley (2005).
[b] Based on central estimates and ranges of number of lymph nodes within region.

Table I.5a. Number and distribution of lymph nodes in the head and neck area[a]

LYMPH NODES IN HEAD AND NECK			Number of lymph nodes[b]								
Occipital			2	-	8						
Mastoid / Retroauricular / Posteriorauricular			4	-	6						
Parotid			10	-	20						
	Superficial parotid					5	-	7			
	Deep parotid					4	-	10			
Facial			10	-	20						
	Malar					NA					
	Mandibular					2	-	4			
	Buccinator					10	-	12			
	Nasolabial					NA					
Submandibular			5	-	8						
Submental			2	-	8						
Retropharyngeal			2	-	4						
	node de Rouviere					NA					
	deep nodes of tongue					NA					
Lateral cervical			55	-	65						
	Deep					40	-	50			
		superior group							18	-	22
		inferior group							20	-	24
		jugulo-omohoid							2	-	4
	Superficial					8	-	16			
Infrahyoid			2	-	4						
Prelaryngeal			5	-	7						
Pretracheal			7	-	9						
Parathracheal			5	-	10						
TOTAL			**120**	**-**	**160**						

[a] Sources: Gray (2000); Agur and Dalley (2005).
[b] Ranges represent either the values found in the two sources or an assigned (rounded) ± 10% if one value was available.

Table I.5b. Number and distribution of lymph nodes in the upper limbs[a]

LYMPH NODES IN UPPER LIMBS	Number of lymph nodes[b]				
Deep axillary nodes	40	-	60		
brachial / lateral group				8	-	12
thoracic / pectoral / anterior group				14	-	18
subcapsular / anterior group				6	-	8
central group				6	-	10
subclavicular / apical group				8	-	12
Superficial nodes	12		16		
cubital / epitrochlear				6	-	10
supratrochlear				4	-	8
TOTAL	**60**	**-**	**70**		

[a] Sources: Gray (2000); Agur and Dalley (2005).
[b] Ranges represent either the values found in the two sources or an assigned (rounded) ± 10% if one value was available.

Table I.5c. Number and distribution of lymph nodes in the thorax[a]

THORACIC LYMPH NODES	Number of lymph nodes[b]					
Parietal nodes	35	-	40			
parasternal				6	-	10
intercoastal				8	-	12
subscapular				10	-	14
deltopectoral				2	-	6
interpectoral				2	-	4
paramammary					NA	
Visceral nodes						
anterior mediastinic						
posterior mediastinic	8	-	12			
bronchial	65	-	75			
tracheo-bronchial						
inferior						2 - 4
superior						5 - 7
tracheal road fork				8	-	14
broncho-pulmonary				6	-	8
intra-pulmonary				40	-	50
arch of azygos vein				2	-	3
aortic arch				2	-	3
TOTAL	**100**	**-**	**130**			

[a] Sources: Gray (2000); Agur and Dalley (2005).
[b] Ranges represent either the values found in the two sources or an assigned (rounded) ± 10% if one value was available.

Table I.5d. Number and distribution of lymph nodes in the abdominal area[a]

ABDOMINAL LYMPH NODES	Number of lymph nodes[b]						
Lumbar, lumbar aortic (parietal nodes)	20	-	30				
right lateral lumbar				8	-	12	
left lateral lumbar				6	-	10	
retro-aortic				4	-	6	
pre-aortic				1	-	5	
Gastric (left and right)	16	-	24				
superior				12	-	18	
upper							
lower							
paracardial							4 - 5
inferior				4	-	7	
gastro-omental							
Epigastric	3	-	4				
Hepatic	15	-	20				
hepatic							
foraminal							8 - 12
cystic							3 - 4
pyloric / subpyloric				6	-	8	
Pancreatic / Splenic							
Pancreatico-lienal / splenic / pancreatic	12	-	16				
Pancreatico-duodenal	6	-	8				
Inferior phrenic	5	-	7				
Celiac	6	-	10				
Superior Mesenteric	130	-	150				
Mesenteric				40	-	50	
along the aortic trunk							
juxtaintestinal							
central superior							
Ileocolic lymph nodes				10	-	20	
upper							4 - 6
lower							8 - 12
Mesocolic lymph nodes				60	-	90	
colic							10 - 20
paracolic							50 - 60
epicolic							
Inferior mesenteric	10	-	20				
left-colic and sigmoid arteries				2	-	4	
sigmoid mesocolon & hemorrhoidal artery				NA			
pararectal (muscle of rectum)				NA			
Appendicular	6	-	8	NA			
TOTAL	**240**	**-**	**280**				

[a] Sources: Gray (2000); Agur and Dalley (2005).
[b] Ranges represent either the values found in the two sources or an assigned (rounded) ± 10% if one value was available.

Table I.5e. Number and distribution of lymph nodes in the pelvis[a]

PELVIC LYMPH NODES			Number of lymph nodes[b]				
Parietal pelvic			30	-	40		
	external iliac					8 - 16	
		intermediate external					NA
		lateral external					NA
		medial external					NA
		inter iliac					NA
		obturator					NA
	internal iliac or hypogastric					8 - 10	
		inferior					NA
		superior					NA
	Sacral					3 - 5	
	illiac circumvent					2 - 4	
	common illiac					4 - 8	
		intermediate					NA
		lateral					NA
		medial					NA
		promotory					NA
		subaortic					NA
Visceral pelvic			6	-	8		
	para-uterine					NA	
	para-vaginal					NA	
	retro-prostatic					3 - 5	
	para-vesicular					NA	
	para-rectal					6 - 8	
	lacunar					NA	
		intermediate					
		lateral					
		medial					
TOTAL			**35**	**-**	**45**		

[a] Sources: Gray (2000); Agur and Dalley (2005).
[b] Ranges represent either the values found in the two sources or an assigned (rounded) ± 10% if one value was available.

Table I.5f. Number and distribution of lymph nodes related to the lower limbs[a]

LYMPH NODES IN LOWER LIMBS			Number of lymph nodes[b]					
Tibial			2	-	4			
	anterior					1	-	2
	posterior					1	-	2
Fibular /peroneal			1	-	3			
Popliteal			4	-	6			
	deep							
	superficial							
Inguinal			25	-	35			
	superficial					15	-	20
		inferior						
		superolateral						
		superomedial						
	deep					10	-	14
		cloquet's node						
TOTAL			**35**	-	**45**			

[a] Sources: Gray (2000); Agur and Dalley (2005).
[b] Ranges represent either the values found in the two sources or an assigned (rounded) ± 10% if one value was available.

The numbers in Tables I.4 and I.5a–I.5f refer to lymph nodes large enough to be shown on anatomical depictions (some as large as a walnut), with clusters found in the armpits, groin, neck, chest and abdomen (near the aorta and in the mesentery) (see also Fig.I.1).

There are thousands of other very small lymph nodes (i.e. as small as a pinhead) that do not have individual names, a rather unusual situation in anatomy (Klein and Horejsi 1997). Using advanced visualization and imaging techniques that were able to show very small nodes (diameter 1–3 mm) in digital images obtained from cross-sections of a human male cadaver (who appears to have been a smoker), Qatarneh et al. (2006) reported finding 1100–1200 nodes in the head and neck, thoracic, abdominal, and pelvic regions, i.e., about twice the number that we estimated for these same regions from graphical depictions and numerical information in conventional atlases of human anatomy (see Table I.4). The diameters of the lymph nodes identified by Qatarneh et al. (2006) ranged from 2–38 mm in the head and neck and thoracic regions, 2–20 mm in the abdomen, and 2–30 mm in the pelvic region.

The information provided by Qatarneh et al. (2006) should not affect our inventory estimates by more than ~1% *if the numbers of lymphocytes within lymph nodes are proportional to the volumes of the nodes* because the data in Tables I.4 and I.5a–I.5f cover the larger lymph nodes (>2–3mm) that are readily visible to the naked eye. However, as noted earlier, there is considerable uncertainty about the relationship between the sizes and immunological activity (hence lymphocyte numbers) of lymph nodes in adult humans. In healthy people, peripheral nodes are inactive while gut-associated lymphoid tissue is highly reactive (MacDonald 2008a).

4.5 Fractions of B Lymphocytes That Could Be CLL Precursors

Lack of information for and uncertainty in potential CLL precursors limits our ability to develop a fully meaningful approach to dose estimation for CLL. Snapshots in time of the distributions of B cells vs T cells are available for many sites (Westermann and Pabst 1992), but there is only scattered information on the specific subsets of B cells that are potential precursors for CLL. Potential precursors were identified in Section 1. They include: (a) circulating V-preB$^+$L$^+$ cells; (b) transitional B cells (which home to the spleen, lymph nodes, or other secondary lymphoid organs and undergo further development into mature antigen-naïve B cells over a period of 3–4 d; Chung et al. 2003); (c) potential human equivalents of the murine B-1

cell;[8] (d) CD5$^+$ follicular mantle B cells; (e) marginal zone B cells (many of which are memory cells; see below); and (f) several types of memory cells, including those which, like marginal zone B cells, have undergone somatic hypermutation outside germinal centers, and without T-cell help (Chiorazzi et al. 2005; Hervé et al. 2005; Chiorazzi 2007). [V-preB$^+$L$^+$ cells are not fully mature even though they have exited the bone marrow to the peripheral blood and transitional B cells are not "antigen-experienced" cells, but neither one can currently be excluded from consideration as CLL precursors (Chiorazzi et al. 2005; Chiorazzi 2007)—in the case of the latter mainly because they express CD5 on the cell surface (see more below).]

The information we obtained may be summarized as follows:

- V-preB$^+$L$^+$ B cells represent only 0.5−1.0% of all circulating B cells (Meffre et al. 2004). Little is currently known about their distribution in secondary lymphoid compartments.

- B cells bearing the CD5 receptor, thought by some to be the human equivalent of murine B-1a lymphocytes, are consistently present in the peripheral blood and spleens of healthy subjects: $17 \pm 5.0\%$ and $17 \pm 3.9\%$ of total B cells, respectively (Casali et al. 1987).However, CD5$^+$ B cells are found most abundantly in the follicular mantle (Chiorazzi 2007). Hardy et al. (1987) cite typical values of 2−3% of total blood lymphocytes and 20−30% of total B cells. Other references suggest more variability: one indicates that in humans "B-1" cells represent 5−10% of B cells in peripheral blood and lymphoid tissues (Abbas and Lichtman 2003), while another says they make up 10−25% of the B-cell pool in blood and lymphoid organs (Morris and Rothstein 1994), and still another indicates that "substantial percentages,

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[8] There may be two distinct lineages of B cells derived from bone marrow, based on studies in mice: B-1 (a or b) and B-2 cells. Stem cells for B-1 cells reportedly disappear from the bone marrow early in life, having given rise to a self-replenishing population of mature B-1 cells, but this may only be true in mice (Morris and Rothstein 1994; Ghia et al. 2007). B-1a cells are CD5$^+$, and B-2 cells are CD5$^-$, although expression of CD5 can be induced later in B-2 cells by some antigens, and all human *transitional* B cells have recently been shown to be CD5$^+$ (Meffre et al. 2004; Cuss et al. 2006). The distinction between B-1 and B-2 cells has been thought to be important because all CLL cells are CD5$^+$, leading some researchers to conclude that CLL arises solely from B-1 cells (Morris and Rothstein 1994; Abbas and Lichtman 2003). B-1 cells express their immunoglobulins from unmutated or minimally mutated germlines (Roitt 2001) and do not participate in germinal center reactions (Paul 2003), making them potential candidates for the precursor of the aggressive form of CLL that also has unmutated or minimally mutated immunoglobulin genes (Chiorazzi et al. 2005). However, recent studies, although not ruling out this possibility, have also failed to provide evidence to confirm the viability of the hypothesis (Hervé et al. 2005). In addition, CD5 is reportedly expressed at variable levels on all human peripheral blood cells and thus cannot be an exclusive marker for the human counterpart of murine B-1a cells (Meffre et al. 2004). Further, Ghia et al. (2007) now suggest that CD5 might be an activation marker rather than a cell lineage feature.

up to 30%, can be found in the peripheral blood and lymphoid nodes" (Kraal 1992). However, it has recently been shown that nearly all human *transitional* B cells express CD5, suggesting that expression of this surface marker does not necessarily characterize "B-1" cells or their equivalent in humans (Meffre et al. 2004; Cuss et al. 2006; van Zelm et al. 2007). In fact, current evidence suggests that most, if not all, B lymphocytes might express the CD5 molecule if properly activated (Ghia et al. 2007). Finally, gene-expression profiling studies (Klein et al. 2001; Rosenwald et al. 2001) did not provide support for the hypothesis that CD5$^+$ B cells could be precursors for either mutated or unmutated CLL.

- Marginal zone B cells are so named because they reside in the marginal zones of the spleen and other lymphoid organs/tissues and are thought by many investigators to develop in the marginal zone of the spleen (Weller et al. 2004; Pillai et al. 2005). They circulate and are found in mucosal-associated lymphoid tissue, lymph nodes, Peyer's patches, and subepithelial areas of tonsils, in addition to the spleen. B-cell receptors of CLL cells are structurally similar to those of antibodies to autoantigens and to Type II T-cell independent antigens (e.g., bacterial polysaccharides) against which marginal zone B cells are particularly active (Brown 1992; Chiorazzi et al. 2005). Up to 30% of the B-cell pool in blood and spleen consists of marginal zone B cells (Weller et al. 2004; Steiniger et al. 2005). Most are reportedly long-lived memory cells, with some surviving the lifespan of the host. Their longevity probably is due in part to constant weak signaling from self antigens and blood-borne pathogens (Pillai et al. 2005).

- Peripheral blood and primary follicles of secondary lymphoid organs are dominated by mature but antigen-naïve B cells throughout life. The half-life of a mature antigen-naïve B cell (as distinguished from a transitional B cell) is estimated to be a few years. In a manner akin to that for marginal zone B cells, survival of these naïve lymphocytes is maintained by weak recognition of self antigens so that cells receive signals that are enough to keep them alive but not enough to activate them to differentiate into plasma or memory cells (Abbas and Lichtman 2003).

- There are 5 or more subsets of B cells, including several different types of memory cells, in human blood (Klein et al. 1998b; van Zelm et al. 2007). Memory cells appear to represent about 25−40% of blood B cells in humans (Klein et al. 1997; Klein et al. 1998a; Weller et al.

2004; Steiniger et al. 2005), with the remainder made up of antigen-naïve B cells, but also including transitional B cells. Klein et al (1998a) found that 40% of the memory cells in human blood express the $IgM^+IgD^+CD27^+$ phenotype considered to be characteristic of circulating splenic marginal zone B cells (Weller et al. 2004; Steiniger et al. 2005; Chiorazzi 2007).

- Memory cells reportedly make up about 70% of bone marrow B cells in adults over 20 y of age (Paramithiotis and Cooper 1997). Some of the latter may actually have been lymphoblasts or long-lived plasma cells, which preferentially home to the bone marrow for growth support by stromal cells (see below), because they were relatively large cells and could be induced to secrete antibody *in vitro*. The fraction of antigen-experienced lymphocytes in all organs/tissues appears to increase with age.

- There are 5−7 different subsets of B cells, including several different types of memory cells, in human tonsils (Liu et al. 1997; van Zelm et al. 2007). About 50−90% of the intraepithelial lymphocytes in the palatine tonsils are B cells, and the majority of the B cells in the crypt epithelium are memory cells (Nave et al. 2001).

- Memory B cells with somatically mutated V genes, generated via the germinal center reaction, have a restricted distribution within lymphoid organs. For example, memory B cells from human tonsils are mainly located within the mucosal epithelium but not in the follicles. They seem to dominate the splenic marginal zones, which are the draining sites of blood-borne antigens (Liu and Arpin 1997), the dome region of Peyer's patches, and the tonsillar subepithelium—all regions that are in close contact to potential entry sites for foreign antigens (Pillai et al. 2005). This intraepithelial localization of memory B cells parallels that of memory T ($CD45RO^+$) cells, which preferentially colonize the skin, the mucosa, and the inflammatory tissues, an apparent strategy to be strategically positioned within antigen-draining sites (Liu et al. 1995). Small B lymphocytes ($CD19^+$: antigen-naïve or memory cells) predominate over plasma cells in Peyer's patches and the appendix, whereas the reverse is seen in the lamina propria, which also appears to contain relatively few memory cells. Notably, $CD19^+$ B cells in the lamina propria are also negative for CD5 (Brandtzaeg and Johansen 2005).

- Less than 0.1% of circulating B-lineage cells are plasma cells (Roitt 2001). Plasma (antibody-producing) cells are not considered to be potential precursors for CLL, but are included here because some authors consider that a set of longer-lived plasma cells (perhaps lymphoblasts) should be considered to be memory cells (McHeyzer-Williams and McHeyzer-Williams 2005). Most intestinal plasma cells, the major pool of antibody-producing cells in the human (Pabst et al. 2008), express somatically mutated (often class-switched) V genes and are thus derived from germinal center reactions (Klein et al. 1998b).

The information on potential CLL precursors has evolved rapidly over the past decade, but there are obviously still many gaps, as well as questions about its current utility. One is left with the impression that the criteria for identification of the different types of CLL precursors (e.g., based on the surface receptor characteristics) is still evolving, such that currently available information may have unforeseen limitations. We were unable to locate quantitative information sufficient to estimate inventories of all of the specific subsets of potential CLL precursors in the organs and tissues with the largest inventories of B lymphocytes in the human body, namely the lymph nodes, spleen, intestinal mucosa, and red bone marrow, with the exception of questionable information about $CD5^+$ cells (as discussed above), some information on marginal zone B-cell inventories in spleen (up to 30% of B cells) and memory cells in the lamina propria, and one estimate of the inventory of memory cells (as a group) in bone marrow (70% of B cells, from Paramithiotis and Cooper 1997). Thus, a detailed tabulation of the available information, similar to that presented in Tables I.1−3, was not warranted.

In 2007, the Office of Compensation Analysis and Support of the National Institute of Occupational Safety and Health (NIOSH) asked several recognized CLL experts to address the question of the most likely B-cell precursor for CLL. Chiorazzi (2007) concluded that since gene expression profiling suggests that unmutated CLL cells do not differ from mutated CLL cells in a large number of differentially expressed genes, the most parsimonious scenario is that both of these subgroups derive from marginal zone B cells. His alternate choice for unmutated CLL was "a heretofore undefined human B-1 cell equivalent" (which would be expected to be $CD5^+$), and for mutated CLL, the human B-2 cell equivalent, through a T-cell dependent germinal center reaction [representing most memory cells], or from $IgM^+IgD^+CD27^+$ memory cells (which he suggests are circulating splenic marginal zone B cells; also see Weller et al. 2004), via a T-cell

independent mutation mechanism occurring outside of classical germinal centers. He thus proposed a possible hierarchy of most likely CLL precursors.

In contrast, Rai opined that "[t]hey are B-lineage lymphocytes which are neither the most primitive…nor fully mature…The CLL lymphocytes have been arrested at a late intermediate stage…If we consider [the] plasma cell as an example of the last and final stage of a normal lymphocyte's differentiation pathway, then [the] CLL lymphocyte has been arrested at a stage just prior to [that]…" Zent (2007) concluded that "the cell of origin of CLL is likely to be a mature B lymphocyte that is antigen experienced and has the gene expression profile of a memory cell even though about half of these have not undergone somatic hypermutation. The lack of somatic hypermutation could possibly indicate drive by a common antigen analogous to a superantigen."[9] [The role of antigen (autoantigen?) stimulation in CLL etiology is currently a major focus of research (Caligaris-Cappio and Ghia 2007; Ghia et al. 2007).]

Based on the opinions expressed by these three experts, we could probably eliminate transitional B cells from consideration. And, a considerable body of information (discussed earlier) also indicates that $CD5^+$ B cells (including follicular mantle B cells) are no longer viable candidates for the CLL precursor, despite Chiorazzi's suggestion that a heretofore unidentified cell from the "B-1" lineage is still a potential candidate. Ghia et al. (2007) indicated that the bulk of scientific opinion is now directed toward the concept that CD5 expression on the CLL cell is not an inherent characteristic of the original B-cell lineage but rather is a product of activation by an encounter with antigen. V-preB^+L^+ B cells cannot currently be eliminated as a possible B-cell precursor because they are "antigen-experienced" and have antibody reactivity characteristics comparable to unmutated CLLs (Hervé et al. 2005). In contrast to Chiorazzi (2007), these authors excluded marginal zone B cells with a phenotype similar to that of circulating $IgM^+IgD^+CD27^+$ memory cells as potential CLL precursors pending determination of their antibody reactivity. Thus, there is currently not a consensus on the part of all CLL experts on how to screen or rank the available candidates for CLL precursors, thus making it difficult to establish a hierarchy of potential candidates.

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[9] Powerful, immunostimulatory, and disease-causing microbial toxins, so-called because of their ability to polyclonally activate large fractions (2−20%) of T cells at extremely low concentrations. They cause a number of diseases characterized by fever and shock and are virulence factors for two human commensal organisms, *Staphylococcus aureus* and *Streptococcus pyogenes*, as well as for some viruses.

The available information indicates that the inventories of potential CLL precursors could range from 0.5% (for circulating V-preB$^+$L$^+$ B cells) to 30% (for marginal zone B cells) of the inventories of B lymphocytes in individual body compartments. However, even this conclusion requires an assumption that the reported numerical distributions for these types of B lymphocytes can be applied to *all* body compartments, even though quantitative data are available only for a limited number of compartments and the distribution of some types (e.g., V-preB$^+$L$^+$ and transitional B cells) is clearly restricted. Given the wide range involved and the fact that expert opinion suggests that antigen-experienced/memory-like cells—and conceivably marginal zone B cells (Chiorazzi 2007)—are the most likely candidates for the CLL precursor (Chiorazzi 2007; Zent 2007), we used a log-triangular distribution to represent the uncertainty in the percentage of CLL precursors in the inventory of B lymphocytes in any given compartment. That is, the calculations included in this report are based on a percentage of CLL precursors described as a log-triangular distribution with a minimum of 0.5%, and a mode and maximum of 30% of the inventories of B lymphocytes in any given compartment.

We took this approach because we did not have enough quantitative information to be able to conduct a formal exercise to assign ranks and weights to the inventories and distributions of the types of B cells identified as the best candidates for potential CLL precursors, e.g., to establish a hierarchy of possibilities, as suggested by Chiorazzi (2007). We have no information currently on the magnitude of the individual weights that should be applied to inventories and distributions for potential precursors and the ranking suggested by different authors are often contradictory.

Using the log-triangular distribution discussed above, we estimated the percentages of total B-lymphocytes represented by potential precursors for CLL using the methods described in Section 3.2. As expected, the resulting estimates (Table I.6) have nearly the same central values as those for percentages of B lymphocytes in body compartments from which they were derived, but with much wider credibility intervals, representing the additional uncertainty associated with our current lack of knowledge about the nature and distribution of potential CLL precursors.

One could argue that the distributions in Table I.6 are a reasonable representation of the uncertainty in the fractional distributions of B-lymphocyte precursors for CLL, because they include both the uncertainties in the fraction of the B lymphocytes that could be CLL precursors and the uncertainties in the compartmental inventories of B lymphocytes as given in Table I.3. A

number of issues that can affect the magnitude of uncertainty in the latter inventories are discussed in the next section of the report.

Table I.6. Estimated percentage distribution of B-CLL precursors in compartments of the human body

Compartments of the human lymphatic system	% of total B-CLL precursors in human body (95% C.I.)[a]
Lymph nodes	27 (2.7−65)
Spleen	23 (2.1−59)
Peyer's patches (small intestinal wall)	3.7 (0.24−14)
Thymus	0.24 (0.010−1.1)
Red bone marrow	18 (1.5−52)
Tonsils (extrathoracic airways)	0.45 (0.018−1.9)
Blood (spleen)	2.3 (0.12−8.7)
Intestinal Mucosa	19 (1.5−56)
Respiratory Mucosa	3.4 (0.20−13)
Skin	0.064 (0.002−0.27)
Liver	0.50 (0.028−1.9)
Vermiform appendix (lower large intestinal wall)	0.036 (0.002−0.14)
Residual soft tissue	1.3 (0.079−4.8)

[a] Based on data from Table I.3 and distribution for uncertainty in B-CLL precursors described in text. Central estimates are means.

5. DISCUSSION OF LIMITATIONS OF EXISTING INFORMATION

Some of the limitations of existing information on inventories and distributions of lymphocytes compiled or synthesized for this report were described in Section 4. Our approach to analysis of the information was designed to deal with some of these limitations, e.g., by making use of calculations or extrapolations to deal with data gaps and expanding uncertainty bounds on estimates where necessary.

This section of the report describes some more general concerns about the quality of the existing information that could affect the credibility of our results, including effects of (1) variations in lymphocyte numbers and distributions with time, (2) lymphocyte circulation, and (3) species-specific differences in anatomy and physiology that could affect information based on extrapolations from data obtained in laboratory animals. It also describes several cases where existing information is questionable or contradictory and discusses potential impacts on our synthesized estimates of lymphocyte inventories and distributions.

5.1 Variation in Lymphocyte Numbers and Proportions with Time

In healthy adult humans, T cells are about twice as numerous as B cells on an overall basis (Westermann and Pabst 1992). However, the ratio of B cells to T cells is highly variable from one lymphocyte pool to another, and this ratio also changes with age and other factors. The composition of the lymphocyte subsets in the organs and their compartments is not static but dynamic. Lymphocytes proliferate in the bone marrow and in a wide variety of tissues, with very large differences in both production rate and lymphocyte output (Westermann and Pabst 1992; Hay and Andrade 1998; Pabst et al. 2008).

- Most of the human data are essentially snapshots taken at one point in time. Yet we know that lymphocyte distribution in humans is not static and can be influenced by many factors: age, gender, race, stress, medication, physical activity, circadian rhythm, and lifestyle factors. In addition, different diagnostic techniques can result in differences in estimates of the normal ranges of lymphocytes in blood, lymph, and other human tissues (Blum and Pabst 2007).

- Numbers, proportions, and distributions of B and T lymphocytes change with age and infectious state (Lazuardi et al. 2005; Blum and Pabst 2007; Sternberg 2007). There are also important changes within the B-cell repertoire itself with advancing age (Sternberg 2007).

- The age dependence of the size and activity of the different parts of the Waldeyer's ring (in particular, the palatine and pharyngeal tonsils; Fig. I.1) is well known but its clinical relevance is only partly understood. In addition, many persons had the palatine and pharyngeal tonsils surgically removed when they were children. Changes in the constitution of the Peyer's patches and appendix were noted earlier in Section 4.1.2.

- The isolated lymphoid follicles in the intestinal mucosa reportedly come and go, depending on the need to respond to infectious agents (Klein and Horejsi 1997).

- In healthy adults, the lymph nodes of the stomach and many more associated with the intestines (see Table I.5d) are hardly identifiable with the naked eye. The inguinal lymph nodes (see Table I.5f) are of palpable size in nearly all age groups, while others except those of the mesentery (see Table I.5d), are only microscopically identifiable (Pabst et al. 2008).

All of these factors add uncertainty to estimates of lymphocyte inventories within specific sites in the human body in ways that cannot currently be quantified.

5.2 Contradictory information on inventory estimates

In several cases, we identified information on inventories of lymphocytes that is potentially contradictory. These are discussed below.

5.2.1 Whole Body

The total number of lymphocytes in humans estimated by both Trepel (1974) and Blum and Pabst (2007) is slightly less than 500×10^9 lymphocytes. However, one apparently authoritative reference reports a total number of 2000×10^9 lymphocytes, with a cell mass equivalent to the mass of the brain or liver, i.e., about 1500 g (Alberts et al. 2002), while the data collected and synthesized by us yield a central estimate of about 570×10^9 lymphocytes, with a range of about $440-740 \times 10^9$ lymphocytes (Table I.3). The high value cited by Alberts et al. (2002) appears to

be traceable to a now-discredited estimate of the lymphocyte inventory and distribution in the human body by Osgood (1954, 1955) (see Trepel 1974). Unfortunately, data from the papers by Osgood were a major source of information for the compilation of information on the human lymphatic system by the ICRP (2002a).

5.2.2 Intestinal Mucosa

Brandtzaeg et al. (2008) indicated that there are *at least* 30,000 isolated lymphoid follicles in the adult human gut, increasing in density distally within each region. The density in the ileum is an order of magnitude higher than in the jejunum, but the density increases only by a factor of three from the ascending colon to the recto-sigmoid region. As discussed earlier, MacDonald (2008a) estimated the total number of follicles to be about 90,000, and he suggested that this value be rounded off to 100,000 because of the increase in body mass of humans since 1965 when one of their primary sources of information on the dimensions of the human gut was published.

Nearly 80% of all plasma cells (the final stage in B-cell differentiation) in healthy adult humans are located in the gut (Brandtzaeg and Johansen 2005; Pabst et al. 2008).[10] Brandtzaeg et al. (1987, 1989) estimated that there were about 10×10^9 antibody-producing cells per meter of human small intestine, thus about 70×10^9 plasma cells in the lamina propria of the small intestine alone (Pabst et al. 2008), or 90×10^9 plasma cells if the content of the small intestine is extrapolated to the entire intestine.

We obtained the same value for the number of lymphocytes in isolated lymphoid follicles of the lamina propria, i.e., not just B cells (those comprising stages prior to plasma cells), but lymphocytes of all types, including T cells which reportedly make up 70% of the total number of lymphocytes in this tissue (Table I.2), by using the estimate of follicle numbers by MacDonald (2008a) and an average of 10^6 lymphocytes per follicle (MacDonald 2008b) (Table I.1). This total does not include the contribution to lymphocyte numbers in the gut from Peyer's patches or from either diffuse lymphocytes within the lamina propria or intra-epithelial lymphocytes (inventories for which have not yet been determined).

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[10] More than 80% of all antibody-producing cells in the mouse were found in the intestinal mucosa, even though they represented only 10% of the lymphocyte pool in that tissue (van der Heijden 1987).

Some gastroenterologists and mucosal immunologists have concluded that the gut harbors at least 70% of *all* lymphoid cells in the human body (390×10^9 lymphocytes, if referenced to our central estimate of the total inventory of lymphocytes in the adult human, plus an estimated 90×10^9 plasma cells), thus representing the largest single lymphoid organ (see discussion in MacDonald 2008a; Pabst et al. 2008, but also Ganusov and De Boer 2008 for a contrary view.)

If we accept this higher estimate, some, or perhaps all, of the following must also be true with respect to our compartmental inventories of lymphocytes: (1) the numbers of lymphocytes in the intestinal mucosa have been grossly underestimated, (2) the numbers of lymphocytes in body compartments other than the intestinal mucosa (e.g., lymph nodes) have been overestimated or (3) the total number of lymphocytes in the human body has been significantly underestimated.

To illustrate: The higher estimate of the numbers of lymphocytes in the gut (390×10^9) is nearly four times larger than our central estimate (100×10^9 cells; Table I.3). Further, if we think that our central estimate of 570×10^9 lymphocytes in the whole body (Table I.3) is reasonable, subtracting the higher estimate of the lymphocyte inventory in the intestinal mucosa leaves a residual of 180×10^9 lymphocytes, which is slightly less than our central estimate for the inventory in lymph nodes alone, thus suggesting that the inventories in lymphoid organs such as the lymph nodes, spleen, red bone marrow, and Peyer's patches could have been greatly overestimated. On the other hand, if the estimates in other lymphocyte-containing body compartments are considered reasonable, we have to increase our estimate of the lymphocyte inventory in the whole body by nearly 70%: $(570-100) \times 10^9 / (1 - 0.7) = 1600 \times 10^9$ lymphocytes to accommodate the higher estimate for the inventory in the gut. The latter is more than twice the value of the upper bound of the 95% credibility interval we estimated for the lymphocyte inventory in the whole body.

Thus, it is conceivable that the uncertainty ranges in the sizes of the lymphocyte pools, not only in the gut but also in other body compartments, could be wider than that indicated in Tables I.1 and I.3. However, we were unable to locate published quantitative data to support the higher estimate of the gut inventory just described. Thus, we elected not use it in establishing our PDF for the lymphocyte inventory in the intestinal mucosa, pending receipt of additional information.

5.2.3 Spleen

As noted earlier, some sources indicate that the relative importance of the spleen might also be greater than we estimated, containing up to 25% of the body's lymphocytes (Brown 1992; Paul 2003). The ultimate source of the higher estimate proved to be a review paper on the immunobiological consequences of splenectomy by Llende et al. (1986). However, that paper does not provide quantitative data to support the estimate, or reference another paper as the source of such data, and we have not been able to locate any other study that provides quantitative data to back up the higher inventory estimate for the spleen. Further, as noted in the previous section, depending on how one interprets information indicating that the gut is the single largest lymphoid organ, current estimates of the lymphocyte inventory in the spleen could be considered overestimates, rather than underestimates. Thus, we have opted not to factor the uncertainty associated with a potentially higher estimate into our PDF for the splenic inventory of lymphocytes at this time.

5.2.4 Peyer's Patches

Klein and Horejsi (1997) reported that there are about 400−600 follicles in Peyer's patches (alternatively, 1000 individual structures, per Paul 2003), mainly in the ileum and but also in the jejunum, and about 200 in the vermiform appendix. The numbers of follicles reported by Klein and Horejsi (1997) and Paul (2003) for the Peyer's patches appear to be significant underestimates. Brandtzaeg et al. (2008) indicate that the number of *Peyer's patches* increases to 250 in the midteens, and then diminishes to about 100 between 70 and 95 years of age. By definition, each Peyer's patch contains at least five follicles, but can contain up to 200 (Brandtzaeg and Johansen 2005), or even more, in particularly large patches, which can exceed 10 cm in length (see Cornes 1965). The average number of lymphoid follicles in the Peyer's patches in an adult human has been estimated to be >5500 (MacDonald 2008a). A complicating factor is that the number of follicles in both Peyer's patches and the appendix decline with age, as noted previously (Cornes 1965; Paul 2003).

5.2.5 Tonsils

There is also considerable uncertainty about the lymphocyte complement of the tonsils. The lingual tonsil is a collection of 35−100 discrete units found at the base of the tongue, each containing several lymphoid follicles (Klein and Horejsi 1997). We have not been able to obtain separate information on the constitution of the pharyngeal or laryngeal tonsils or the paired palatine and tubal tonsils, other than an estimated amount of tissue approximately 2−3 cm in diameter based on tonsillectomy specimens (MacDonald 2008a). As discussed earlier, we had to resort to graphical and other descriptive information to estimate lymphocyte inventories in the tonsils as a whole. There is also significant age-related variation in these structures (Section 5.1). This information led us to use a larger range estimate (spanning an order of magnitude) for the lymphocyte inventory in tonsils in Table I.1.

5. 3 Effects of Lymphocyte Movements

The peripheral blood contains 20−50% of circulating lymphocytes; the remainder move within the lymphatic system (Alberts et al. 2002). Each day roughly 500×10^9 lymphocytes migrate from the blood into the lymphoid tissue throughout the body of a young adult human, approximately the same as the number of lymphocytes within the whole body as estimated based on the data presented in Table I.1. The mean blood transit time is 25 ± 6 min, resulting in an exchange rate of 48 times per d (Pabst 1988; Westermann and Pabst 1992).

In contrast, the daily recovery of lymphocytes from the thoracic duct in humans is only about 30×10^9 lymphocytes, about 6% of the daily emigration from the blood, so most must leave the blood by routes other than the lymph nodes. Half leave the blood via the spleen, which is the single most important lymphoid organ in lymphocyte recirculation, surpassing all of the lymph nodes put together by about an order of magnitude (Pabst 1988), as well as the body's principal line of defense against bloodborne antigens (Kraal 1992).

Based on studies in sheep, about 60% of the blood lymphocytes constituted the recirculating pool, which is characterized by the ability of the cells to exit the blood and circulate through the lymphatic system back into the blood. This pool was dominated by T lymphocytes, with only

25% B cells. In contrast, the non-recirculating blood pool (40% of the total), which consists of cells that remain within the blood system, contained 85% B cells (Blum and Pabst 2007).

Thus, the recirculating pool cannot be regarded as homogeneous, as it is composed of multiple populations of lymphocytes with different behaviors. The distinction between "recirculating" and "sessile" or fixed lymphocytes is also unclear. Hay and Andrade (1998) have asked whether there really are lymphocytes that remain fixed in lymphoid tissues. Answering their question could shed light on an important issue for radiation dosimetry, namely whether an assumption that a B lymphocyte might take up residence at a fixed location for a long period of time could be valid. Antibody-producing (plasma) cells (which are neither lymphocytes *per se* nor CLL precursors) seem to have a more sessile existence once they have homed to their designated tissue site, e.g., bone marrow, intestinal mucosa, or infection site, but memory B lymphocytes clearly have some capacity to recirculate.

Prenodal (afferent) lymph contains a lower proportion of B cells than postnodal (efferent) lymph (see Table I.2) but a higher proportion of memory cells. However, it also appears that memory cells are concentrated in areas such as the splenic marginal zone or other antigen draining sites where the circulation can bring antigens into contact with them. Presumably the nonrandom assortment of lymphocytes in these various compartments increases the efficiency of the immune response by localizing cells with particular roles to the sites where they are most required (Hay and Andrade 1998).

As noted earlier, an assumption inherent in our approach to estimating a meaningful internal radiation dose for CLL in Part II of this report is that the lymphatic system *can be treated as a system in dynamic equilibrium*, namely that lymphocyte distributions and inventories in all compartments of the human body are always in a steady-state condition. That assumption is challenged by the existence of significant temporal variations (e,g., in response to antigens) and the age-related changes discussed earlier that are not currently reflected in our estimates. Current information is inadequate to estimate the relative importance of these temporal variations to radiation dose estimates. However, the uncertainty ranges currently assigned to the inventories and fractions of B lymphocytes may be sufficiently wide to capture the effects of temporal variations, i.e., because not all data were obtained from "single snapshots in time" and wide credibility intervals were assigned to those that were.

5.4 Effects of Species-Specific Differences

There are also important differences in the lymphoid organs or the organization of the lymphatic system among different species, a potentially important concern when having to rely in part on extrapolations of data from animals to humans (Pabst and Binns 1986; Brandtzaeg et al. 2008). For example, Brandtzaeg and Johansen (2005) pointed out that our knowledge of the human mucosal B-cell system is blurred because mechanistic information is largely based on animal experiments. Structural characteristics and microenvironments within human lymphoid organs are often different than those in the animals in which most of the studies of the lymphatic system have been performed (rodents, pigs, and sheep; see Pabst and Binns 1986; Kraal 1992; Steiniger et al. 2005), adding to the uncertainty in data based on extrapolations and raising additional issues for internal dosimetry in humans. There are particularly significant differences among species in the structure and function of the spleen, for example, which is the largest discrete lymphoid organ (Brown 1992; Paul 2003). Marginal zone B cells are found only in the spleen in rodents, but in humans they circulate and are found in several other sites, as noted earlier.

Pabst et al. (2008) also point out that the typical pathogen-free conditions of many laboratory animals do not mimic the situation in humans who are exposed to a continuous change in microbial composition bombarding mucosal surfaces and a non-standardized food intake.

With only a few exceptions where it was necessary to complement human data with animal data (see Sect. 4.1), the information accumulated for this report was based on human data.

6. SUMMARY AND CONCLUSIONS

Current information indicates that CLL is produced by transformation of mature, antigen-experienced B lymphocytes, possibly memory cells, potentially anywhere in the body (i.e., including but not restricted to the bone marrow). This situation complicates an assessment of the risk of developing CLL of radiogenic origin because definition of an appropriate target organ or tissue is problematic because radiation doses from internally deposited radionuclides and also from some types of external exposures can be very different at different locations within the body. Thus, B cells at different sites could receive markedly different doses.

Resolution of the problem therefore appears to require a probabilistic solution. Namely, to be meaningful for assessment of the risk of developing radiogenic CLL, the radiation dose should be a weighted average based on both the dose to a given site and the probability that a B lymphocyte (more properly a B-cell precursor for CLL) will occupy that site.

Part I of this report presents the results of a literature search and synthesis of information on inventories and distributions of B lymphocytes, including potential CLL precursors, to try to meet the stated need. Available information on inventories of lymphocytes (i.e., all types combined) and fractions of the inventories that are B lymphocytes was used in combination to estimate compartment-level inventories of B lymphocytes in as many individual compartments of the human body as the data permitted. Because information about the fractions of B lymphocytes that could be CLL precursors was quite limited, compartment-level inventories of these B cells were derived using a relatively simple approach.

The intent is to use this compartment-level information (expressed as percentages of the total inventory of B lymphocytes or CLL precursors in the body) as weights in an inventory-weighted calculation of average radiation dose. Options for applying this information to the stated purpose are examined in Part II of this report, along with two less-complicated but also less realistic approaches for estimating radiation doses that could be used in risk assessment for CLL.

Body compartments containing significant amounts of lymphocytes include lymph nodes, spleen, intestinal mucosa, thymus and red bone marrow, with others being of secondary importance (Table I.3). However, <1% of the lymphocytes in thymus are B cells. Thus, the largest inventories of B lymphocytes in the human body reside in the lymph nodes, spleen, intestinal mucosa, and red bone marrow. These inventories are roughly an order of magnitude

larger than those in the Peyer's patches, respiratory mucosa, peripheral blood, and our "residual soft tissue" grouping, which includes muscle tissue, lymph, body cavity fluids, the greater omentum, and mucosa of the urogenital tract. Other "sites" have inventories that are two or more orders of magnitude less than the latter grouping (Table I.3).

Most of these estimates have large uncertainties, and they also may not reflect the average behavior of the B lymphocyte complement during adulthood because they are based on information that often represents a snapshot in time. As noted earlier, an assumption inherent in our approach to estimating a meaningful internal radiation dose for CLL in Part II of this report is that the lymphatic system *can be treated as a system in dynamic equilibrium*, namely that lymphocyte distributions and inventories in all compartments of the human body are always in a steady-state condition. It could be argued that this assumption is invalidated by the existence of significant temporal variations (e,g., in response to antigens) and age-related changes that are not explicitly accounted for in our inventory estimates. However, it is also conceivable that the uncertainty ranges currently assigned to inventories and fractions of B lymphocytes are sufficiently wide to capture the effects of such temporal variations, i.e., because not all data were obtained from "single snapshots in time" and wide credibility intervals were assigned to those that were.

Of equal importance, the available information on inventories and distribution of the half-dozen individual types of B lymphocytes that are currently considered potential precursors for CLL is currently too limited to attempt a compilation of the type shown in Table I.3 for B lymphocytes as a group.

One option for dealing with the lack of information on potential B-cell precursors for CLL is to assume that they are all distributed in the same proportions as other B lymphocytes among all lymphocyte-containing body compartments, despite the existence of information to the contrary. These difficulties cannot be overcome by focusing on just one potential precursor (e.g., a specific type of antigen-experienced/memory-like cell) because there are several different subsets of such cells to consider. Questions about their proportions and distributions within specific secondary lymphoid organs or tissues, the mucosa, in particular, would remain, and the uncertainty about whether a memory cell (and if so, which memory cell) was the appropriate precursor would still have to be dealt with.

These questions and uncertainties, along with differences of opinion among CLL experts, currently prevent us from conducting a formal exercise to rank and weight the inventories and distributions of B cells suggested as the best candidates for CLL precursors, e.g., to establish a hierarchy of possibilities, with marginal zone B cells at the top of the hierarchy as suggested by Chiorazzi (2007). We have no information currently on the magnitude of the weights that should be applied to inventories and distributions of potential precursors.

These limitations in current information led us to the lymphocyte distributions in Table I.6, which were based on the application of a wide range of uncertainty in the fractional distributions of potential CLL precursors to the inventories of B lymphocytes in Table I.3 to derive inventories and uncertainty estimates for the CLL precursors. Namely, it is assumed that all B-lymphocyte precursors for CLL are found in all compartments and that fractional distributions of all B-lymphocyte precursors for CLL have identical and equally wide uncertainty bands. One could argue that the distributions in Table I.6 are a reasonable representation of the uncertainty in the fractional distributions of B-lymphocyte precursors for CLL, because they include both the uncertainties in the fraction of the B lymphocytes that could be CLL precursors and in the compartmental inventories of B lymphocytes given in Table I.3.

By applying Monte Carlo methods of uncertainty propagation, the outcomes of interest in risk estimation will reflect the uncertainties in the dose estimates, which, in turn, are determined in large part by the widths of the ranges for compartment-level percentages of potential B-cell precursors for CLL. However, some individual types of precursors may be restricted in their distribution throughout the body and some may have much narrower ranges than the assigned uncertainty bands; the effects of these factors on our proposed compartmented-weighted dose-averaging scheme have not been investigated at this time.

7. UNRESOLVED QUESTIONS

Our study has provided information on the human lymphatic system that is potentially useful for defining a meaningful radiation dose for assessing the radiological risk of CLL, but it also has prompted many questions, many of which may require additional research to provide the answers:

- Have we incorporated uncertainties appropriate to deal with the current lack of information on the half-dozen types of potential CLL precursors? Could the uncertainties be reduced by ranking and weighting the inventories and distributions of specific types of potential precursors, e.g., to establish a hierarchy of possibilities, as suggested by Chiorazzi (2007)? Does the uncertainty introduced by temporal and age-related variability in lymphocyte inventories and distribution need to be considered formally? What level of effort would be required to determine whether the effects of this variation on radiation dose estimates are negligible or significant? Some simplified modeling may be able to tell us whether this effect is likely to be large or not. To what extent would such estimates have to be tailored to account for the route of entry of a radionuclide into the body and internal kinetics and partitioning specific to a given radionuclide/solubility class? The last question will be addressed in Part II of this report. The others will require input in the form of peer review from technical experts in immunology and radiation dosimetry and/or additional study to address.

- If the dose to a mature B lymphocyte determines the biological response to radiation exposure, but that B cell potentially could have arisen at any time during the life of an individual diagnosed with CLL *and could only be affected by radiation exposure after it matured*, does the term "radiation dose" as it is normally used in conjunction with a specific population of susceptible cells within a target organ or tissue have any meaning? How does one define important parameters in radiation risk models such as "age at exposure" or "time since exposure" under such conditions? To what extent does irradiation of stem and progenitor cells of the lymphocytes *in the bone marrow* influence later development of CLL,[11] even though the event that transforms the normal B cell to a CLL clone occurs at a

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[11] An inherited familial predisposition to CLL has been documented (Keating et al. 2003; Chiorazzi et al. 2005; Linet et al. 2007; Rai 2007) and genetic differences in immunoglobulin genes considered relevant to CLL are

later time and in a different location? These questions appear to require additional clinical

and laboratory research to provide the answers.

observed in populations at low risk for developing CLL (e.g., persons of far East Asian ancestry; Keating et al. 2003). Low incidence rates for CLL persist in individuals migrating to the U.S. from Asian countries and in their descendants (Linet et al. 2007). Thus, it is conceivable that irradiation could produce genetic changes in bone marrow stem cells that could result in increased susceptibility to development of CLL that might only be manifested at later stages of lymphocyte development.

PART II APPLICATION TO RADIATION DOSIMETRY FOR CLL

1. INTRODUCTION

In this part of the report, the information we developed on lymphocyte inventories and distributions is applied to the problem of how to estimate a meaningful radiation dose for CLL, i.e., one that accounts for the energy delivered by radiation to the entire CLL precursor population, which is potentially scattered throughout the human body. An *average* radiation dose to the cells of a target organ or tissue is normally used for estimates of radiogenic cancer risk (e.g., for lung or liver cancer) (ICRP 1991). Thus, we begin by examining the possibility of defining an average dose among all compartments of the body that contain potential CLL precursors. The average is obtained by weighting the doses to different compartments according to the fractions of B lymphocytes or potential B-lymphocyte precursors for CLL defined by the data in the rightmost column of Table I.3 and in Table I.6, respectively, in Part I of this report. Section 2 of this part of the report illustrates how information about such distributions of B lymphocytes might be used to estimate such an average dose.

Because of questions about the validity of these distributions of B lymphocytes, we also examined two other less complicated but less realistic options for dose estimation: (1) use of the maximum radiation dose to any compartment containing potential B-lymphocyte precursors for CLL and (2) a log-uniform distribution of radiation doses, ranging from the minimum dose to the maximum dose to any such compartment. Despite their simplicity, each of these options also has some significant drawbacks (see Sections 3 and 4).

The four options are compared and discussed with respect to their potential applicability for risk assessment for CLL, including their advantages and disadvantages with respect to their ability to account for current uncertainties in the underlying information (Section 5).

2. INVENTORY-WEIGHTED AVERAGE DOSE DISTRIBUTIONS

In this approach, estimates of radiation doses received by each lymphocyte-containing body compartment are multiplied by the estimated fraction of the total body inventory of B-lymphocytes, either for potential precursors for CLL in that compartment, as in Table II.1, which

Table II.1. Estimated distribution of B-CLL precursors in compartments of the human body and information for calculation of radiation doses based on unit intakes of inhaled insoluble plutonium-239 or ingested soluble strontium-90

Compartments of the human lymphatic system (radiation doses assigned)	% of total B-CLL precursors in human body (95% C.I.)[a]	Dose to compartment for unit intake of Pu-239 (Sv/Bq)[b]	Dose to compartment for unit intake of Sr-90 (Sv/Bq)[c]
Lymph nodes	27 (2.7−65)		
Extrathoracic; fraction of nodes = 0.05−0.07[d]		7.50E-05	6.60E-10
Thoracic; fraction of nodes = 0.07−0.09[d]		**8.20E-04**	6.60E-10
Remainder; fraction of nodes = 0.84−0.88[d] (majority of residual soft tissues; see below)		2.90E-07	6.60E-10
Spleen	23 (2.1−59)	2.90E-07	6.60E-10
Peyer's patches (small intestinal wall)	3.7 (0.24−14)	2.90E-07	8.00E-10
Thymus	0.24 (0.010−1.1)	2.90E-07	6.60E-10
Red bone marrow	18 (1.5−52)	8.50E-06	**1.80E-07**
Tonsils (extrathoracic airways)	0.45 (0.018−1.9)	4.20E-05	6.60E-10
Blood (spleen)	2.3 (0.12−8.7)	2.90E-07	6.60E-10
Intestinal Mucosa	19 (1.5−56)		
(small intestinal wall; fraction of mucosa = 0.8[e])		2.90E-07	8.00E-10
(upper large intestinal wall; fraction of mucosa = 0.1[e])		3.00E-07	1.30E-9
(lower large intestinal wall; fraction of mucosa = 0.1[e])		3.10E-07	1.80E-9
Respiratory Mucosa	3.4 (0.20−13)		
(extrathoracic airways; fraction of mucosa <0.001[f])		4.20E-05	6.60E-10
(lung; fraction of mucosa >0.999[f])		7.90E-05	6.60E-10
Skin	0.064 (0.002−0.27)	2.90E-07	6.60E-10
Liver	0.50 (0.028−1.9)	3.60E-05	6.60E-10
Vermiform appendix (lower large intestinal wall)	0.036 (0.002−0.14)	3.10E-07	1.80E-9
Residual soft tissue[g]	1.3 (0.079−4.8)		
(adrenals, breast, esophagus, muscle, pancreas, thyroid, uterus, "remainder;" fraction of residual by mass = 0.98)		**2.90E-07**[h]	**6.60E-10**[i]
(bladder wall; fraction of residual = 0.002)		2.90E-07	1.50E-09
(kidneys; fraction of residual = 0.009)		7.30E-07	6.60E-10
(ovaries; fraction of residual = 0.0003)		2.20E-06	6.60E-10
(stomach wall; fraction of residual = 0.005)		2.90E-07	9.00E-10
(testes; fraction of residual = 0.001)		2.30E-06	6.60E-10

Footnotes for Table II.1

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[a] Based on information in Table I.6 in Part I. Central estimates are means.

[b] 50-y committed doses for the case of insoluble plutonium-239 (aerodynamic median aerosol diameter 1.0 micron) inhaled by an adult radiation worker, obtained from ICRP (2002b).

[c] 50-y committed doses for the case of soluble strontium-90 ingested by an adult radiation worker, obtained from ICRP (2002b) for all organs/tissues other than the intestines and from Figure 8.1 in ICRP (2006) for the intestines.

[d] Estimated from numbers of nodes draining nasopharyngeal and tracheal regions (~40) in Tables I.5a and I.5c and bronchopulmonary and intrapulmonary regions (~50) in Table I.5c compared with total numbers of lymph nodes in body given in Table I.4. The ICRP (1994) estimated that the mass of the extrathoracic and thoracic lymph nodes was the same.

[e] Based on lengths of small and large intestine given in footnote h to Table I.1 and equal lengths of upper and lower large intestine reported in ICRP (2002a).

[f] Based on either relative masses or surface areas; see ICRP (2002a).

[g] For purposes of radiation dosimetry, this compartment includes: adrenals, bladder wall, breast, esophagus, kidneys, muscle, ovaries, pancreas, prostate, stomach wall, testes, thyroid, uterus, and "remainder"as defined by the ICRP (2002b). Its total mass is estimated as 33 kg for an adult male [lean body mass minus the mass of the skeleton, brain, and the specific compartments of the lymphatic system (e.g., lymph nodes, blood, etc.) listed in the rows above].

[h] The dose for unit intake of the "remainder" compartment is 3.20E-07 Sv/Bq, but this difference has a negligible effect on the integrated dose for the other compartments listed.

[i] The dose for unit intake of the "remainder" compartment is 6.70E-010 Sv/Bq, but this difference has a negligible effect on the integrated dose for the other compartments listed.

was derived from information in Table I.6 of Part I of this report or for B lymphocytes *in toto*, as in Table I.3 of Part I. An integrated dose estimate covering all body compartments is obtained by summing the adjusted doses for all compartments. For internal exposures, this approach is specific for each radionuclide (and solubility class) and route of entry.

2.1 For Potential B-Lymphocyte Precursors for CLL

The first set of calculations uses as input the information for fractional distributions of potential B-lymphocyte precursors for CLL (Table II.1). This approach assumes that a single distribution for the fraction of B lymphocytes that are potential precursors for CLL can be applied to the B-lymphocyte inventories in all body compartments, even though quantitative data are available only for a limited number of compartments and some potential precursors are known to have restricted distributions (see discussion in Section 4.5 in Part I).

Our first test case using this approach involves estimation of an average weighted dose resulting from inhalation of an insoluble form of plutonium-239. The lymphocyte-containing compartment receiving the highest dose from inhaled insoluble Pu-239 is the collection of thoracic lymph nodes (Table II.1).

To ensure that our results are not an artifact of the choice of radionuclide, solubility class, and route of entry into the body, we have also calculated an integrated unit dose for ingestion of soluble Sr-90. The lymphocyte-containing organ receiving the highest dose from ingested soluble Sr-90 is the red bone marrow (Table II.1).

To perform our calculations, we obtained estimates of doses to each body compartment for unit intakes of plutonium-239 and strontium-90 from the ICRP (2002b, 2006) (see Table II.1). These radionuclides are reasonable choices because they both have long physical and biological half-lives and accumulate preferentially in some body compartments, leading to highly non-uniform dose distributions (Table II.1).

Because there is not a complete one-to-one correspondence between body compartments for which radiation doses are estimated and those for which we have estimates of inventories of CLL precursors, some interpolation is necessary (see Table II.1 and footnotes d-g):

- The fractions of thoracic and extrathoracic lymph nodes for purposes of dose assignments were estimated by comparing the numbers of nodes draining (1) nasopharyngeal and tracheal

regions (~40) in Tables I.5a and I.5c of Part I of this report and (2) bronchopulmonary and intrapulmonary regions (~50) in Table I.5c of Part I, respectively, with the total numbers of lymph nodes in the body given in Table I.4 (also from Part I). The ICRP (1994) estimated that the mass of the extrathoracic and thoracic lymph nodes was the same.

- The fractional weights assigned to the regions of intestinal wall for dose assignments were based on relative lengths of small and large intestine given in footnote h to Table I.1 of Part 1 of this report and on equal lengths of upper and lower large intestine, as reported in ICRP (2002a).

- Another issue is that the doses given by the ICRP (2002b, 2006) for the intestinal wall may be overestimates for the lamina propria (which is estimated to contain the bulk of the gut's lymphocytes) in some cases because they apply to exposures of the gut epithelia. The effects on doses from plutonium-239 are expected to be minimal since they are based on systemic contributions to doses (i.e., no contribution from direct irradiation by gut contents) and are very similar to the doses delivered to most other soft tissues (ICRP 2006). The effects on our calculations for the case involving strontium-90 have not been fully determined yet. Doses from soluble strontium-90 are result from both exposure of the intestinal wall by beta particles emitted from gut contents during passage through the intestines and internal exposure from radioactivity redistributed following absorption through the gut wall (ICRP 2006). However, we think that the effects are likely to be small because the largest contribution to the dose appears to result from internally redistributed material.

- The fractions of the respiratory mucosa used to assign radiation doses to the lung and to the extrathoracic airway were based on relative masses and relative surface areas; the fraction assigned to the lung was 0.999 in either case (see ICRP 2002a).

- For purposes of dose assignment and apportionment, the "residual soft tissue" compartment includes: adrenals, bladder wall, breast, esophagus, kidneys, muscle, ovaries, pancreas, prostate, stomach wall, testes, thyroid, uterus, and "remainder" as defined by the ICRP (2002b). Its total mass is estimated as 33 kg for an adult male [lean body mass minus the mass of the skeleton, brain, and the specific compartments of the lymphatic system (e.g., lymph nodes, blood, etc.) listed in the rows above "residual soft tissue" in Table II.1].

Relative masses were used to assign weights to groupings or individual organs or tissues within this grouping (see Table II.1).

Multiplying each fractional compartmental distribution of B-lymphocyte precursors for CLL (with uncertainties) by the unit dose to that compartment and, where necessary, the fractional weights for assigning the doses to sub-compartments just described, using Monte Carlo methods, and summing the results provides an integrated weighted estimate of unit dose for purposes of estimating the probability of causation of CLL from radiation exposure. This multiplication was repeated 2000 times for each Monte Carlo sample.

Probability distributions for the inventory-weighted average doses obtained for the cases of Pu-239 inhalation and Sr-90 ingestion are shown in Table II.2 and Fig. II.1.

2.2 For B Lymphocytes as a Group

The second set of calculations uses information for B-lymphocytes as a group, i.e., by substituting the fractional inventories in the rightmost column of data in Table I.3 of Part I of this report for those in Table II.1 above. The rest of the information utilized is identical to that used in the first set of calculations (i.e., fractional weights and dose assignments for lymphocyte-containing body compartments and corresponding unit doses for inhalation of insoluble plutonium-239 and ingestion of soluble Sr-90; see Table II.1). The number of iterations (2000 times) and approach to Monte Carlo sampling were also the same.

Table II.2 and Fig. II.1 also provide probability distributions for these inventory-weighted average doses, again for the two cases of Pu-239 inhalation and Sr-90 ingestion. As expected, the distributions are narrower and the upper bounds are lower than in the distributions weighted by compartment-specific inventories of potential CLL precursors.

**3. UNWEIGHTED LOG-UNIFORM DISTRIBUTION OF DOSES**

Another option is a radionuclide-specific log-uniform distribution ranging from the lowest to the highest doses estimated by the ICRP (2002b, 2006) for the body compartments in which potential B lymphocyte precursors for CLL reside (see values in bold face in Table II.1). For

Table II.2. Estimated radiation doses potentially relevant to risk assessment for chronic lymphocytic leukemia in adult workers, from unit intakes of inhaled insoluble plutonium-239 or ingested soluble strontium-90

| Radionuclide (estimation method) | 50-y committed dose (Sv/Bq) | | | | | | Maximum |
| | Percentiles of dose distribution estimated by Monte Carlo methods | | | | | | |
	2.5	5.0	50	Mean	95	97.5	
Pu-239 (B-lymphocyte inventory-weighted average dose distribution)[a]	1.3E-05	1.3E-05	1.9E-05	2.0E-05	2.7E-05	2.8E-05	
Pu-239 (CLL-precursor inventory-weighted average dose distribution)[a]	5.0E-06	6.0E-06	1.8E-05	1.9E-05	3.7E-05	4.0E-05	
Pu-239 (log-uniform dose distribution)[b]	3.5E-07	4.3E-07	1.5E-05	1.0E-04	5.5E-04	6.7E-04	
Pu-239 (maximum dose to any compartment)							8.2E-04
Sr-90 (B-lymphocyte inventory-weighted average dose distribution)[a]	1.5E-08	1.7E-08	3.3E-08	3.4E-08	5.4E-08	5.8E-08	
Sr-90 (CLL-precursor inventory-weighted average dose distribution)[a]	3.2E-09	4.2E-09	2.8E-08	3.4E-08	8.5E-08	9.9E-08	
Sr-90 (log-uniform dose distribution)[b]	7.6E-10	8.7E-10	1.1E-08	3.2E-8	1.4E-07	1.6E-07	
Sr-90 (maximum dose to any compartment)							1.8E-07

[a] Based on multiplication of fractional distributions of lymphocytes and unit doses in Table II.1 or the alternative distributions for B lymphocytes *in toto* described in Section 2.2; distribution created using Monte Carlo sampling techniques (2000 Latin Hypercube samples).
[b] Based on range of doses in Table II.1; distribution created using Monte Carlo sampling techniques (2000 Latin Hypercube samples).
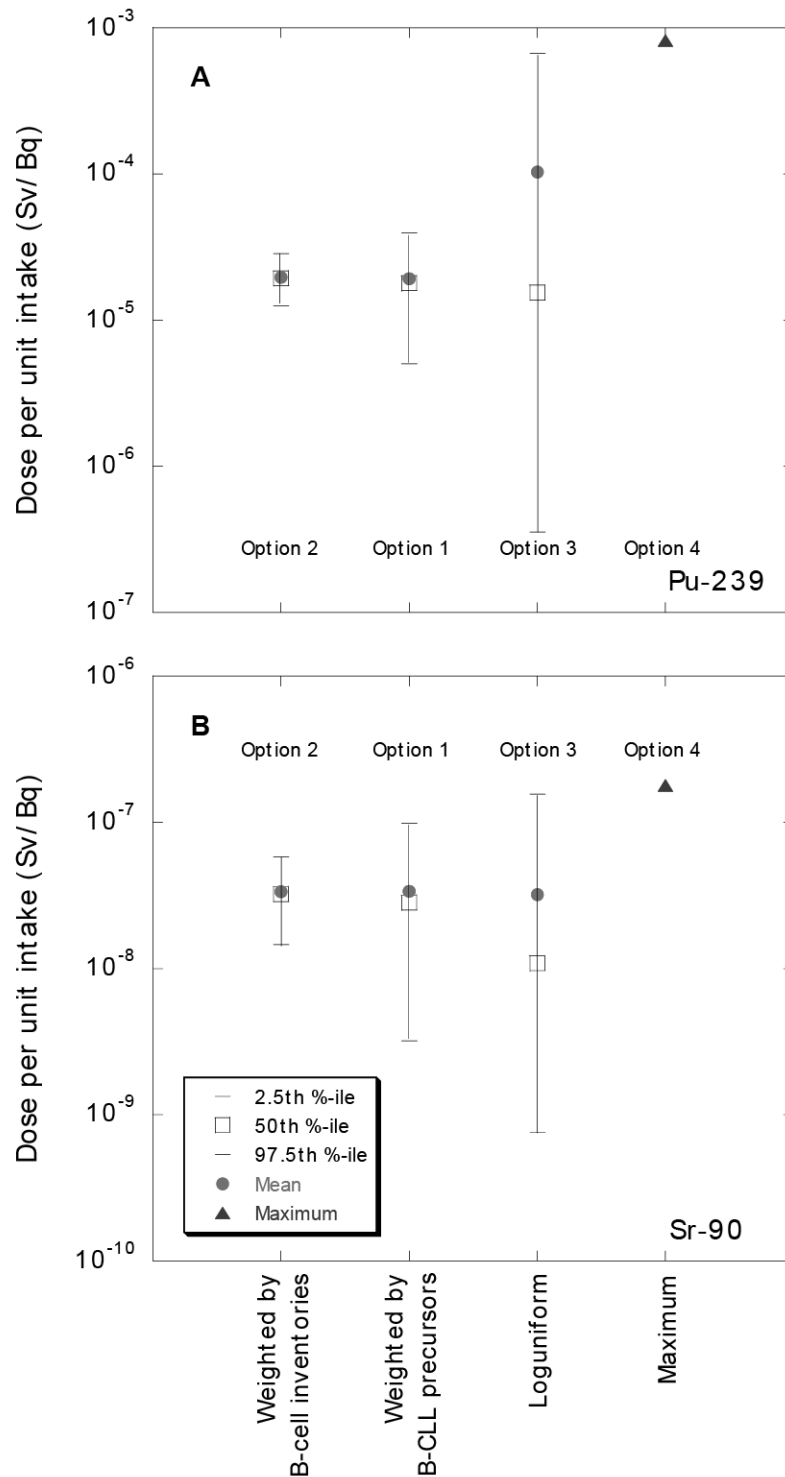
Fig. II.1. Radiation doses from unit intakes of inhaled insoluble plutonium-239 or ingested soluble strontium-90 for estimation of radiologic risk of chronic lymphocytic leukemia in adult workers.

internal exposures, this approach also requires information on the route of entry into the body (ingestion, inhalation, or wound) and solubility class, as in the previous cases. This distribution assumes complete lack of knowledge about the distribution of these CLL precursors within these compartments.

In the case of inhalation of insoluble plutonium-239 (aerodynamic median aerosol diameter 1.0 micron) inhaled by an adult radiation worker, the ICRP (2002b) estimates that the unit dose to the thoracic lymph nodes received by age 70 would be 2.8×10^3 times that received by the spleen (one of several organs or tissues that received the lowest doses; Table II.1). Thus, the distribution of potential doses for this case runs from the logarithm of the dose to the spleen to the logarithm of 2.8×10^3 times that dose, with equal probabilities assigned to all doses within that range.

Doses were selected from within this range by Monte Carlo random sampling techniques (2000 iterations) to create a log-normal distribution of doses that could be used to estimate the risk for developing radiogenic CLL. The same approach was applied to the information for strontium-90 in Table II.1.

These results, once again for the two cases of Pu-239 inhalation and Sr-90 ingestion, are also shown in Table II.2 and in Fig.II.1. The unweighted lognormal distribution is the widest of those considered and has the highest upper bounds on radiation dose resulting from exposure to either radionuclide. It is based on an assumption of complete ignorance about the lymphatic system, one that appears to make it scientifically indefensible.

4. MAXIMUM DOSE TO ANY LYMPHOCYTE-CONTAINING ORGAN OR TISSUE

A fourth approach for dealing with the current limited information (and the list of unresolved questions in Section 7 of Part I) would be to simply select the highest dose received by one of the four body compartments that contain the largest inventories of B lymphocytes, namely the lymph nodes, spleen, intestinal mucosa, and red bone marrow, as the relevant dose for induction of CLL. For the lymph nodes, regional doses could be assigned when appropriate. For internal exposures, this approach also requires information on the route of entry into the body (ingestion, inhalation, or wound) and solubility class, as in the three previous cases considered.

This approach has the appeal that it potentially yields the highest risk but it too is unrealistic and appears to be scientifically indefensible.

One obvious problem with such an approach is that radiation doses to lymphocyte-containing organs or tissues[12] with relatively small B-cell inventories, such as the liver, can be much greater than those to the thoracic lymph nodes, spleen, intestinal mucosa, and red bone marrow in some cases (e.g., lifetime doses following inhalation of *soluble* forms of plutonium; ICRP 2002b). Such sites cannot be excluded from consideration *a priori* because of current uncertainty about the site where a B lymphocyte precursor for CLL was located at the time it was transformed to a CLL clone.

For the two test cases we considered (inhaled insoluble plutonium-239 and ingested soluble strontium-90), this particular concern does not apply, because the body compartments receiving the highest unit doses are the thoracic lymph nodes and red bone marrow, respectively (Tables II.1 and II.2).

However, another concern—and the one that led us to pursue the compartment-weighted average dose option—is that the likelihood that a B lymphocyte will receive the highest dose depends on the *probability* that it has resided at the location receiving the highest dose. And that probability is a function of the relative proportion of the body's B lymphocytes (particularly the CLL precursors) which occupy that location. Thus, sites such as the thoracic lymph nodes[13] or the liver would be expected to contain a relatively small proportion of the total number of B lymphocytes (or CLL precursors) in the human body. Hence, the low probability that a B-lymphocyte precursor for CLL would be located in one of the thoracic lymph nodes (or, alternately, the liver, for the case of soluble plutonium) should reduce the likelihood that it would be one of the cells receiving the high doses delivered to cells occupying that site.

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[12] This term is somewhat of a misnomer since virtually all organs or tissues contain some lymphocytes. It is meant to exclude tissues such as bone surfaces for which radiation doses may be much higher than for other organs or tissues in some cases of internal exposure but which are not expected to contain significant numbers of lymphocytes.

[13] The masses of the thoracic and extrathoracic lymph nodes in adults (15 g each) are reportedly about 2−2.5% of that of the mass of the lymphatic system as a whole (600−730 g, depending on gender) and about 0.025−0.04% of the lean body mass (ICRP 1994, 2002a).

5. COMPARISON OF APPROACHES

This part of the report introduced four options for estimation of radiation doses potentially relevant for assessment of the risk of CLL:

- Option 1 – An average dose (and its uncertainty) obtained using weights based on the fractional distribution of B-lymphocyte precursors for CLL

- Option 2 – An average dose (and its uncertainty) obtained using weights based on the fractional distribution of all B lymphocytes in different compartments of the lymphatic system

- Option 3 – A log-normal distribution of doses ranging from the lowest to the highest possible doses among body compartments in which B-lymphocyte precursors for CLL reside.

- Option 4 – A fixed dose equal to the maximum possible dose among body compartments in which B-lymphocyte precursors for CLL reside.

Options 1 and 2 are referred to as inventory-weighted average doses. The results for all four options are presented side-by side in Table II.2 and Fig. II.1 for the two radiation exposure regimes considered.

The uncertainty in the estimated dose to the population of cells of interest is highest for Option 3 (the unweighted log-uniform distribution of doses). This option assumes no knowledge about the distribution within the lymphatic system of cells of interest for induction of CLL, namely that the true but unknown dose to the population of cells of interest must only fall somewhere between the minimum and the maximum doses among all body compartments containing potential CLL precursors.

There is no uncertainty associated with Option 4, which is simply a "conservative" approach in the sense that the highest dose to any portion of the lymphatic system is selected to be used in the assessment of risk of CLL. Option 4 also assumes no knowledge about the distribution of the cells of interest for induction of CLL and it does not represent an average dose to the population of cells of interest (i.e., potential precursors can be located in body compartment other than the one receiving the highest dose).

Options 3 and 4 are attractive because they are simple, but they are probably less realistic than Options 1 and 2 which try to account for the information (limited as it may be) about the lymphatic system relevant to induction of CLL.

Our analysis includes two approaches in which doses to different portions of the lymphatic system are weighted by the inventories of cells potentially responsible for induction of CLL. In Option 2 the weighting is done using the estimated inventory of B lymphocytes in each compartment of the lymphatic system. Option 1, on the other hand, attempts to account for the fact that only some subsets of B lymphocytes are potential CLL precursors, while acknowledging that there is only limited information (i.e. there is a large uncertainty) about (a) which B lymphocytes are CLL precursors, (b) where these precursors are located, and (c) how they are moving or change with age. Both Option 1 and Option 2 account for the uncertainties related to the inventory of B lymphocytes in each compartment of the lymphatic system (see Tables I.3 and I.6 in Part I of this report), but only Option 1 accounts for the additional uncertainty due to the lack of knowledge about which of the B cells are CLL precursors. That is, the smaller uncertainty associated with Option 2 (see Fig.II.1) appears to reflect overconfidence generated by ignoring the fact that only some of B cells are CLL precursors.

Although the inventory-weighted approaches seemingly provide a more realistic estimate of radiation dose for estimating the probability of causation of CLL than the other options, they still do not capture the full complexity of the lymphatic system. Neither do they address issues associated with other important questions raised in Section 7 of Part I of this report. Whether these uncertainties are enough to offset the differences between the inventory-weighted approaches and the unweighted log-uniform distribution is difficult to judge based on current information, but is currently considered unlikely.

The inventory-weighted average dose based on information for potential CLL precursors (i.e., Option 1) appears to best reflect the nature of and uncertainties in current information about the lymphatic system—and thus is judged to represent the most scientifically defensible option.

If we were to assign the highest radiation dose received by *any* lymphocyte-containing organ or tissue (Option 4)—or the unweighted log-uniform distribution (Option 3)—to estimate a dose for CLL induction, we would be ignoring the (admittedly incomplete) information that we already have, namely that the probability that a B lymphocyte will occupy a given site must vary according to the fraction of B lymphocytes (strictly B-lymphocyte precursors) that occupy that

site. Thus, there is little support for an option that simply assigns the highest dose to any lymphocyte-containing organ or tissue to estimate a meaningful radiation dose for CLL. Such an approach appears to us to be overly conservative; there appears to be enough information currently available to argue against that choice on technical grounds.

6. CONCLUDING THOUGHTS

In spite of some misgivings about the nature of the current information on the lymphatic system and its applicability to dose estimation, an inventory-weighted average dose based on information for potential CLL precursors appears to be the most scientifically defensible option at the present time. It appears to be infeasible to attempt a more comprehensive exercise in estimating internal radiation doses for CLL until more definitive information on its specific B-cell precursor(s) and their distribution in the human body becomes available. Compilation of the information in this white paper is thus only one step toward determining how to develop an appropriate dosimetric model for CLL (or other non-Hodgkins lymphomas) of radiogenic origin. Taking the next steps appears to require additional clinical and laboratory research to provide the missing information identified in Sections 6 and 7 of Part I of this report.

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