Rat lungs show a biphasic formation of new alveoli during postnatal development

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Tschanz SA, Salm LA, Roth-Kleiner M, Barré SF, Burri PH, Schittny JC. Rat lungs show a biphasic formation of new alveoli during postnatal development. J Appl Physiol 117: 89–95, 2014. First published April 24, 2014; doi:10.1152/japplphysiol.01355.2013.—Roughly 90% of the gas-exchange surface is formed by alveolarization of the lungs. To the best of our knowledge, the formation of new alveoli has been followed in rats only by means of morphological description or interpretation of semiquantitative data until now. Therefore, we estimated the number of alveoli in rat lungs between postnatal days 4 and 60 by unambiguously counting the alveolar openings. We observed a bulk formation of new alveoli between days 4 and 21 (1.74 times increase from 0.8 to 14.3 million) and a second phase of continued alveolarization between days 21 and 60 (1.3 times increase to 19.3 million). The (number weighted) mean volume of the alveoli decreases during the phase of bulk alveolarization from ~593,000 μm3 at day 4 to ~141,000 μm3 at day 21, but increases again to ~298,000 μm3 at day 60. We conclude that the “bulk alveolarization” correlates with the mechanism of classical alveolarization (alveolarization before the microvascular maturation is completed) and that the “continued alveolarization” follows three proposed mechanisms of late alveolarization (alveolarization after microvascular maturation). The biphasic pattern is more evident for the increase in alveolar number than for the formation of new alveolar septa (estimated as the length of the free septal edge). Furthermore, a striking negative correlation between the estimated alveolar size and published data on retention of nanoparticles was detected.

late alveolarization; continued alveolarization; bulk alveolarization; alveolarization; microvascular maturation; stereology; disector; lung development

THE STRUCTURAL PROPERTIES of the lung are well suited for its function as gas exchanger. For optimal diffusion, a maximized surface area of the interface and a minimized barrier thickness between the two phases—air and blood—are needed and achieved by an ingenious and delicate architecture of parenchymal lung tissue. The key functional elements of the lung are the alveoli and their delimiting interalveolar septa. The number and geometry of alveoli essentially influence the amount of available gas-exchange surface capillaries, thus affecting the diffusion capacity of the lung.

Start of alveolarization. At birth, the gas-exchange units in the rat lung consist exclusively of sacculi, which are delimited by rather thick but smooth inter-“saccular” walls, the “primary septa.” A few days after birth, numerous small ridges arise from the saccular wall, the so-called “secondary septa,” which grow in a centripetal manner into the sacculi in order to subdivide them into alveoli (4). At this stage, both primary and secondary septa show a double capillary layer and are termed “primitive” or “immature” septa, as opposed to the mature ones in adult lungs. The human lung shows already at birth such secondary septa, but the main phase of septation happens clearly postnatally (46). Shortly after birth (day 4) the morphology of ongoing septation does not allow to distinguish between sacculi and alveoli.

End of alveolarization. Burri et al. (4, 6) postulated that the formation of secondary septa requires a double-layered capillary network in the preexisting septum from which the new septum is lifted off. Each side has its “private” capillary bed. This structural condition allows an unfolding of the capillary network of one septal side, still ensuring blood supply of the other side. Furthermore, after the maturation of the alveolar microvasculature from a double-layered to a single-layered capillary network, the formation of new alveoli was not detected anymore in developmental studies performed by morphological observation of lung sections. Consequently, it was assumed that after microvascular maturation has completed the formation of new alveolus/new alveoli is discontinued. Until recently, it was not possible to verify or falsify this latter hypothesis, because the required imaging tools [synchrotron radiation-based X-ray tomographic microscopy (33)] and the required quantitative methods [the estimation of the number of alveoli (20, 26) or the estimation of the length of the free septal
edge (33)) were not available. As soon as these tools had been developed it turned out that new alveoli are formed until young adulthood, as shown by now for Rhesus monkeys, rats, mice, and humans (10, 19, 23, 25, 33). Using high-resolution synchrotron radiation-based X-ray tomographic microscopy we (33) showed recently that for alveolarization the postulated requirement of a double-layered capillary network is still valid and that, if necessary, the required capillaries may be newly formed locally by angiogenesis. It is well accepted that the formation of new alveolar septa continues at least until young adulthood.

Aim of study. In a previous study we (33) quantitatively investigated postnatal lung development of the rats, focusing on the formation of alveolar septa. Alveolarization was assessed by tracking septation in terms of lengthening of the free septal edges, which represents the formation of new alveolar walls.

The aim of this study was to directly elucidate the time course of alveolarization by an unbiased estimation of the alveolar number during postnatal rat lung development and to compare it to our previously obtained rat data. In contrast to the developmental increase of the length of the free septal edge (33), we observed a much more pronounced biphasic formation of new alveoli.

MATERIALS AND METHODS

Animals and lung tissue preparation. Male Sprague-Dawley rats were lactated until weaning at about day 21 and afterwards fed with standard rat pellets and water ad libitum. The animal experiments were approved by the Swiss Federal Act on Animal Protection and the Swiss Animal Protection Ordinance.

At each of postnatal days 4, 10, 21, 36, and 60, five animals were taken for lung fixation. After deep anesthesia a pneumothorax was applied and lungs immediately fixed in situ by intratracheal instillation of 2.5% glutaraldehyde in potassium phosphate-buffered solution at a constant pressure of 20 cmH2O column (42). The pressure in the lungs was maintained for at least 24 h. Thereafter the volumes of the separated lung lobes were assessed by water displacement (31) and the total lung volume calculated. Shrinkage due to embedding was achieved by using the disector principle based on pairs of sections (37). “New” appearing septal bridges from an image on one section to the corresponding image on the consecutive section were counted, and the unbiased counting of connections (“bridges”) in space from sections requires a three-dimensional sample. This was achieved by using the disector principle based on pairs of sections (37). “New” appearing septal bridges from an image on one section to the corresponding image on the consecutive section were counted, and this resulted directly in the number of alveoli per probe volume. In order to get the absolute number of alveoli the alveolar density was multiplied by the reference volume, that is, the parenchymal density (entire lung minus pleural tissue and vessels/bronchi larger than 25 μm) times the lung volume (shrinkage corrected), according to the following formula:

\[ N_{alv} = \left( \frac{\sum \text{bridges}}{\text{V}_{\text{disectors}}} \right) \times V_{\text{parenchyma}} \times V_{\text{lung}} \times \text{(shrinkage factor)} \]

where \( N_{alv} \) is the number of alveoli, \( V_{\text{disectors}} \) is the total volume of all dissectors, \( V_{\text{parenchyma}} \) is the volume density of lung parenchyma, and \( V_{\text{lung}} \) is total lung volume.

This approach is design-based and free of any assumptions on shape, size, or distribution of alveoli. The distance of section pairs set at 4 μm was realized by taking consecutive sections of a thickness of 4 μm (26). The calculated coefficient of error depending on the number of estimated alveoli per animal was ~7%. Compared with the observed overall coefficients of variations from 10 to 20%, the stereological approach appears to be precise enough.

The animals used were part of our studies about the pulmonary effects of a neonatal high-dose short-term glucocorticoid treatment of rats (11, 22, 29, 30, 34, 42). In addition, the controls of this experiments were used in other studies analyzing lung development (14–16, 27, 33, 43).

Section sampling. The right middle lobe of each animal was embedded in paraffin. Earlier studies have indicated that in rats one entire lobe represents a valid sample for the whole lung (45). With respect to alveolar structures lung parenchyma can be considered as isotropic (18); therefore isotropic uniform random sections were cut perpendicular to the longitudinal axis of the lobe without special attention to orientation. Sections were cut at a thickness of 4 μm, stained with fuchsin, and numbered. Approximately 10 section pairs equidistantly spaced over the whole lobe were used for stereology. Image pairs were recorded and analyzed using the newCAST software (Visiopharm, Hørsholm, Denmark) and a 20× lens on an Olympus AH-2 light microscope (Olympus Schweiz AG, Volketswil, Switzerland).

Alveolar number. Alveolar number was assessed in the lung parenchyma by counting the rings shaping the alveolar openings (alveolar entrance rings) (20, 26). These rings are part of a three-dimensional network. According to its Euler characteristics, every additional connection within the network represents an additional ring and thus one alveolus. The unbiased counting of connections (“bridges”) in space from sections requires a three-dimensional sample. This was achieved by using the disector principle based on pairs of sections (37). “New” appearing septal bridges from an image on one section to the corresponding image on the consecutive section were counted, and this resulted directly in the number of alveoli per probe volume. In order to get the absolute number of alveoli the alveolar density was multiplied by the reference volume, that is, the parenchymal density (entire lung minus pleural tissue and vessels/bronchi larger than 25 μm) times the lung volume (shrinkage corrected), according to the following formula:

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where \( N_{alv} \) is the number of alveoli, \( V_{\text{disectors}} \) is the total volume of all dissectors, \( V_{\text{parenchyma}} \) is the volume density of lung parenchyma, and \( V_{\text{lung}} \) is total lung volume.
Alveolar volume and mean volume of alveoli. Within the lung parenchyma the volume of alveolar air space, alveolar septa, and alveolar duct air space was assessed. The latter comprised all acinar air spaces centripetal of the alveoli as no distinction between alveolar sacs and ducts was made. On the sections used for number estimation, images were captured with a LEICA DMRB light microscope using a 10× objective by means of a systematic uniform random sampling. Volume density was assessed by point counting using the STEPanizer stereology tool (www.stepanizer.com) (40). Absolute volumes of alveolar and alveolar duct spaces were computed as above using parenchymal density and lung volume:

\[
V_x = V_v \times V'_{\text{parenchyma}} \times V_{\text{lung}}
\]

where \(V_x\) is the absolute volume of the subcomponent \(x\), \(V_v\) is the volume density of the subcomponent \(x\), \(V'_{\text{parenchyma}}\) is the volume density of lung parenchyma, and \(V_{\text{lung}}\) is total lung volume. Here no shrinkage correction was needed as the lung volume before embedding (water displacement) was multiplied by dimensionless volume density estimates not affected by shrinkage.

Mean number weighted volume of an alveolus was calculated by dividing the absolute alveolar volume by the total number of alveoli:

\[
mV_{\text{alv}} = \frac{(V_{\text{alv}} \times V'_{\text{parenchyma}} \times V_{\text{lung}})}{N_{\text{alv}}}
\]

where \(mV_{\text{alv}}\) is the mean number weighted volume of an alveolus, \(V_{\text{alv}}\) is the volume density of alveolar air space, \(V'_{\text{parenchyma}}\) is the volume density of lung parenchyma, \(V_{\text{lung}}\) is total lung volume, and \(N_{\text{alv}}\) is the number of alveoli.

For variance analysis one-way ANOVA and post hoc tests based on the Bonferroni correction for multiple comparisons were performed.

Synchrotron radiation-based X-ray tomography. Lung samples, obtained as described above, were embedded in paraffin and scanned by a monochromatic X-ray beam (12.398 keV) at the micromotography station of the Materials Science Beamline at the Swiss Light Source (Paul-Scherrer-Institute, Villigen, Switzerland) (29). For 3D visualization and surface rendering we applied the software Imaris (Bitplane AG, Zürich, Switzerland).

RESULTS

Volume of parenchymal compartments. The parenchymal volume was categorized into the following volumetric parts: 1) alveolar volume: air space volume surrounded largely by interalveolar walls, 2) ductal volume lying in between alveoli, and 3) septal volume of lung parenchyma (see Table 1 and Fig. 2).

Figure 2 shows the progression of the various volume fractions in the observed period. All components enlarged significantly until the end of the observation period. The net enlargement of ductal (871%) and septal volume (802%) tended to parallel total lung volume (891%) from days 4 to 60 while the volume increase of alveolar space by 1,061% in the same period was disproportionately higher. The pattern of volume increase was slightly different in the three fractions. Septal volume density (data not shown) had its peak at day 10 (28.8%) with a significant drop to 20.3% toward day 21 and staying on the lower level until the end of the experiment. Thus septal volume had the lowest increase rate from days 10 to 21 (+43%), while air space volumes (ductal and alveolar) showed maximal gains (+125%).

Alveolar number. At the beginning of the observation period at day 4, some \(0.82 \times 10^6\) alveoli were counted. However, at this early maturation state, it was not evident to distinguish between original sacculi and “true” newly formed alveoli with our quantification method (see Introduction). At day 21, \(14.3 \times 10^6\) alveoli were present and at day 60 there were \(19.3 \times 10^6\). The progression curve of alveolar number during the observation (Fig. 3) showed a characteristic biphasic shape with a steep increase from day 4 to day 21 (\(0.82 \times 10^6\) to \(14.3 \times 10^6\), \(+1,640\%\)). From days 21 to 60 the number increased further and significantly, but less steeply (+35%) to \(19.3 \times 10^6\).

Mean volume of alveoli. The size of alveoli varied considerably during the observation period (Figs. 4 and 5). However, as mentioned previously, at day 4 the estimation procedure for

Table 1. Body weight, volumes, and number of alveoli

<table>
<thead>
<tr>
<th></th>
<th>Body Wt, g</th>
<th>Lung Volume, cm³</th>
<th>Alveolar Airspace Volume, cm³</th>
<th>Ductal Airspace Volume, cm³</th>
<th>No. of Alveoli, × 10⁶</th>
<th>Mean Volume of Alveolus, μm³</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Day 4</td>
<td>13.1</td>
<td>0.8</td>
<td>1.03</td>
<td>0.07</td>
<td>0.486</td>
<td>0.073</td>
</tr>
<tr>
<td>Day 10</td>
<td>27.0*</td>
<td>3.8</td>
<td>1.81*</td>
<td>0.23</td>
<td>0.880*</td>
<td>0.117</td>
</tr>
<tr>
<td>Day 21</td>
<td>79.2*</td>
<td>4.5</td>
<td>3.61*</td>
<td>0.34</td>
<td>1.910*</td>
<td>0.154</td>
</tr>
<tr>
<td>Day 36</td>
<td>209.0*</td>
<td>8.9</td>
<td>7.28*</td>
<td>0.49</td>
<td>3.956*</td>
<td>0.360</td>
</tr>
<tr>
<td>Day 60</td>
<td>418.0*</td>
<td>24.9</td>
<td>10.21*</td>
<td>0.59</td>
<td>5.645*</td>
<td>0.323</td>
</tr>
</tbody>
</table>

Values are means and SD from \(n = 5\) animals. Significance: *\(P < 0.05\) to the preceding day. †\(P < 0.05\) day 60 vs. day 21.
the parenchymal airspaces did not allow to distinguish between sacculi and alveoli. This also explains why the observed “airspace” volume was 2.2 times larger ($0.59 \times 10^6 \text{mm}^3$) than the alveoli at day 10 ($0.27 \times 10^6 \text{mm}^3$). The smallest mean alveolar volume was found at day 21, being only $0.14 \times 10^6 \text{mm}^3$. At day 60 the mean volume reached $0.30 \times 10^6 \text{mm}^3$.

DISCUSSION

The characteristic structural subunits of the mammalian lung are the alveoli. The number of alveoli, as well as the rate and time course of alveolar formation, are important parameters that define the maturational and developmental state of the lung and its function after growth. The schedule of lung maturation has been well described in rats (4, 6), mice (2), and humans (39, 46, 47) by means of quantitative and morphological observations. In these studies, however, unbiased alveolar numbers have never been obtained. Usually the outgrowth of new septa was taken as the sign for the onset of alveolar formation, while the end of alveolarization was inferred from the degree of septal maturation and from the missing of new septal buds. As an alternative to the estimation of the number of alveoli (quantification of alveolarization) the formation of new alveolar septa (quantification of septation) was followed throughout postnatal lung development in rats and mice by means of estimation of the length of the free septal edge (23, 33). The length of the free septal edge corresponded to the total length of the alveolar entrance rings. Both studies showed that new septa are formed until young adulthood, but the precise correlation to the formation of the alveoli remained open.

The present study defines for the first time the timetable of alveolar formation by a direct and unbiased assessment of the number of alveoli in the rat model. Starting at postnatal day 4 with only $0.82 \times 10^6$ alveoli (or more correctly sacculi) the number increased to $3.5 \times 10^6$ at day 10 and almost $14.3 \times 10^6$ at day 21. This means that within 17 days $13.5 \times 10^6$ alveoli were newly formed what represents a rate of 800,000/day (or 33,000/h). In this period lung volume increased less

Fig. 3. Increase of the total number of alveoli with age. Increase in alveolar number is biphasic. During the first phase (bulk alveolarization) a large increase was observed (days 4–21). Afterwards a second phase of slower but significant increase follows (continued alveolarization). Significance level of differences between days: *$P < 0.05$ ($n = 5$). Error bars indicate ± 1 SD.

Fig. 4. Number weighted mean volume of alveoli during postnatal lung development. During postnatal development the mean size of the alveoli decreases until day 21. Afterwards it increases again, but does not reach the mean size of the sacculi. Significance level of differences between days: *$P < 0.05$ ($n = 5$). Error bars indicate ± 1 SD.

Fig. 5. Visualization of the changing size of the alveoli during rat lung development. Comparing the size of the alveoli (sacculi at day 4) bulk alveolarization causes a dramatic decrease of the size of the airspaces starting from day 4 (A) via day 10 (B) and up to day 21 (C). During continued alveolarization the increase of the lung volume is larger than the increase of the number of alveoli. As a result the size of the alveoli increases between days 21 (C) and 60 (D). The 3-dimensional visualization was done after synchrotron radiation-based X-ray tomographic microscopy. The boundaries of individual sacculi (day 4, A) and alveoli (days 10–60, B–D) are labeled with a dotted line. Bar, 50 μm (without accounting for shrinkage; after correction for the shrinkage the bar represents 35.8 μm).
than four times, which hints at the enormous transformation of the inner lung structure. Alveolarization corresponds to a phenomenal increase in gas-exchange surface area (6). In the 39 days from day 21 to day 60 only $5 \times 10^8$ alveoli were added, clearly underlining the continuation of alveolarization. The mean rate of alveoli formed in this second or late phase, assuming a theoretical linear increase, dropped to 128,000 per day at what corresponds to roughly one-sixth of the rate in the early phase. However, these rates are just theoretical values not taking into account any slow-down of formation. The recognition of a biphasic behavior of alveolarization with the term of “bulk alveolarization” for the first phase and “continued alveolarization” for the second phase is of high developmental and clinical importance.

Just at the beginning of alveolarization the size of airspaces, still being predominantly sacculi, is at its highest level. During the first phase of alveolar formation (days 4–10), when there is a transition from sacculi to alveoli, the mean individual volume of alveoli dropped heavily. Septation is so intense that the volumetric expansion of the alveolar air space does not follow, and therefore the alveoli are becoming much smaller. An inverse situation appears in the phase of “continued” alveolarization when the formation of new alveoli is less important than the growth of the alveoli, leading to an increase of the mean airspace volume.

Between days 21 and 36, the minute increase of alveolar number and the following increase to day 60 can be interpreted as a transitional period where early “bulk” alveolarization has faded out and continued alveolarization is not as efficient to be detected. As lung volume still increases considerably between days 21 and 36 the mean size of alveoli increases, too.

While the formation of new alveoli during the early or “bulk” phase is morphologically well described, the question arises which potential mechanisms allow new alveoli to be formed in the phase of continued alveolarization, where the microvascular pattern is that of a mature lung. Three mechanisms were proposed so far (5, 33). First, the formation of new alveolar septa at sites where the alveolar microvasculature never fully matured, and therefore the required double-layered capillary network still exists [ca. 5–10% of the capillary network (30)]. To be exact this first mode represents ongoing classical alveolarization.

Second, at sites of mature single-layered capillary networks a focal and temporary reduplication of the capillary bed can establish conditions for septal upfolding. Such reduplication was explicitly found in vascular casts at locations where new interalveolar septa started to emerge (33). The second mode can operate everywhere in the lung parenchyma. In a rat model alveolarization was experimentally depressed by various glucocorticoid regimes. After the treatment immature septa with two capillary layers reappeared and a marked recovery of alveolarization was found (30, 41). This might be an enhanced form of the second mode of late alveolarization.

Third, at the parenchymal border where alveoli neighbor nonparenchymal structures, i.e., next to bronchi, vessels, and pleural tissue, alveolar walls have their own, private capillary bed, and it was postulated that an upfolding of new septa may take place throughout life at these sites (5). The latter represents the only kind of alveolarization that does not require a double-layered capillary network, because this capillary network exchanges the gas only at one side and not at two opposing sides like the capillary networks of mature alveolar septa. It is postulated that all of the three modes of late alveolarization take place throughout postnatal lung development after maturation of most alveolar septa.

All listed mechanisms fulfill the requirement of a “private” capillary bed per septal side where one side intends to fold up and form a new interalveolar septum. Our finding of a continued alveolarization after the completion of microvascular maturation could provide an explanation for the lung’s potential to functionally and structurally adapt to environmental changes, like hypoxia (8, 9, 28), and to recover following resection of lung tissue. Functional recovery of the lung was shown to take place after pneumonectomy and other clinical/experimental situations for mice, rats, dogs, and humans (3, 7, 10, 12, 17, 24, 38, 44). This means that the lung is not only capable of partially restoring the diffusion surface area by expanding its airspaces but also by forming new ones. Even in lungs showing a stable compartmental volume and/or alveolar structure, a constant remodeling of the parenchyma without changing the net number of alveoli cannot be excluded.

The two different phases of alveolarization are not only distinguished in morphological investigation, but also observed during the studies of protein expressions, e.g., while tenasin-C is strongly expressed close to the free septal edge during bulk alveolarization, the tenasin-C expression is reduced to a weak and diffuse expression during continued alveolarization. An early short-term treatment with dexamethasone (postnatal days 1–4) did not only postpone and prolong bulk alveolarization, but also the strong expression of tenasin-C was shifted to later days (29). The expression of elastin, which is at least essential for the bulk alveolarization (21), does not change towards or during continued alveolarization (29). The latter may be taken as evidence that elastin is essential for any kind of pulmonary alveolarization.

Semmler-Behnke et al. (35) showed that the deposition of nanoparticles in infant rat lungs shows a peak at postnatal day 21, at the end of bulk alveolarization (35). Comparing their deposition pattern with our data describing the size of the alveoli a striking negative correlation between these two data-sets is observed. Between days 4 and 21, we estimated a decrease of the alveolar volume by a factor of 4, which correlates to the observed five times increase of the deposition of 20-nm particles. While between days 21 and 36 (or 60), the size of the alveoli roughly doubled, roughly half of the deposition was reported comparing days 21 and 36 (or 60). The negative correlation with our results quantitatively validates the computational simulations of nanoparticle retention of Semmler-Behnke et al. (35), where they predicted that the deposition of nanoparticles depends on the size of the alveoli and that small alveoli cause a higher retention.

In our study the mean number of alveoli present in rats at day 21 was $14.3 \pm 3.1 \times 10^6$. This is more than half of the $34 \times 10^6$ estimated using a model-based approximation by Burri et al. (6). At day 60, at the end of adolescence, we observed $19.2 \pm 3.1 \times 10^6$ alveoli which corresponds to $20 \times 10^6$ alveoli published by Hyde et al. (20) in adult rats using the dissector counting principle, too. Using a dissector-based approach on virtual sections from X-ray tomographic scans of lungs obtained from the same rats (42) but studying only the medial lower edge of the right lower lung lobe, Haberthür et al. (14) estimated 46.3 million alveoli in 60-day-old rats (14).
twofold difference may be explained as follows. First, for technical reasons, Haberthür et al. did not use a sampling that represents an entire lung lobe. Regional differences inside one lung lobe, be it biological variability or an uneven filling of the lung, may contribute to the different numbers of alveoli. Second, Haberthür et al. used a shrinkage factor which resulted in half of the shrinkage as the one we observed. However, in both studies the shrinking factor was determined without obvious error using the same stereological methods. The only difference was that the lungs Haberthür et al. use were stored in fixative of 15 years, and our material was embedded directly after the tissue was obtained and stored for the same time as paraffin blocks.

With respect to mean alveolar volume the differences between our smaller volume at day 60 (0.30 ± 0.04 × 10⁶ µm³) and the values of Hyde et al. (0.5 ± 0.18 × 10⁶ µm³) can be explained with the higher body weight and lung volume in the probably older rats (no age information) in the work of Hyde et al. (20). A similar increase was observed comparing the total alveolar volume (our day 60 rats, 5.6 ± 0.3 cm³; those of Hyde et al., 8.5 ± 1.98 cm³). The disproportionate volume increase with only slight number increase of the number of alveoli explains the higher mean volume of alveoli in the work of Hyde et al.

Figure 6 compares key parameters of alveolarization. Alveolarization shows a strong biphasic behavior with a fast formation of alveoli before day 21 (~73% of the absolute number increase) and a slow one afterwards (~27%). This phenomenon is much stronger for alveolarization than for the increase in the total length of the free septal edge. Based on the growth of the lung parenchyma and the total length of the free septal edge, the anlage of new alveolar septa may be calculated (33). The disproportionate volume increase with only slight number increase of the number of alveoli explains the higher mean volume of alveoli in the work of Hyde et al.

In summary we would like to conclude from our unbiased quantitative estimation of the alveolar number that the early phase of alveolarization corresponds to the known mass (“bulk”) production rate of alveoli, which is in good agreement with the morphological findings (4, 6). Because most of the alveolar microvasculature is still immature in this phase (days 4–21), the alveoli will be predominately formed according to the mechanism of classical alveolarization. After day 21 alveolarization continues at a slower pace and follows the three possible mechanisms of late alveolarization, discussed above. Therefore, we would like to call this latter phase continued alveolarization.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

REFERENCES